

HUMAN TUMOR NECROSIS FACTOR RECEPTOR TR16

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This Application, which claims benefit under 35 U.S.C. § 119(e) based on Provisional Application Serial No. 60/268,364, filed February 14, 2001, is a Continuation-In-Part of, and claims benefit under 35 U.S.C. § 120 of copending U.S. Application Serial No. 09/637,856, filed August 10, 2000, which claims benefit under 35 U.S.C. § 119(e) based on Provisional Applications, Serial Nos. 60/148,348, 60/148,683, 60/148,758, 60/148,870, 60/149,181, 60/149,453, and 60/149,498 filed August 12, 1999, August 13, 1999, August 16, 1999, August 13, 1999, August 17, 1999, August 18, 1999, and August 19, 1999 respectively, each of which Applications is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to TR16, a novel member of the tumor necrosis factor family of receptors. More specifically, isolated nucleic acid molecules are provided encoding TR16 splice variants, TR16-short and TR16-long, (herein collectively referred to as TR16 or TR16 receptor). TR16-short and TR16-long polypeptides are also provided, as are vectors, host cells, and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of TR16 activity.

BACKGROUND OF THE INVENTION

[0003] Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intra-cellular response.

[0004] For example, tumor necrosis factors (TNF) alpha and beta are cytokines, which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-

ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

[0005] Among the ligands there are included TNF-alpha lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-2-beta), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and nerve growth factor (NGF). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (A. Meager, *Biologicals* 22:291-295 (1994)).

[0006] Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (A. Meager, *supra*).

[0007] Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (R. Watanabe-Fukunaga *et al.*, *Nature* 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (R.C. Allen *et al.*, *Science* 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innervation of peripheral structures (K.F. Lee *et al.*, *Cell* 69:737 (1992)).

[0008] TNF alpha and LT-alpha are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-alpha acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF alpha and LT-alpha are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmune disease, AIDS and graft-host rejection (B. Beutler and C. Von Huffel, *Science* 264:667-668 (1994)). Mutations in the p55 receptor cause increased susceptibility to microbial infection.

[0009] Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (p55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia *et al.*, *Cell* 74:845 (1993)).

[0010] Apoptosis, or programmed cell death, is a physiologic process essential to the normal development and homeostasis of multicellular organisms (H. Steller, *Science* 267:1445-1449 (1995)). Derangements of apoptosis contribute to the pathogenesis of several human diseases including cancer, neurodegenerative disorders, and acquired immune deficiency syndrome (C.B. Thompson, *Science* 267:1456-1462 (1995)). Recently, much attention has focused on the signal transduction and biological function of two cell surface death receptors, Fas/APO-1 and TNFR-1 (J.L. Cleveland *et al.*, *Cell* 81:479-482 (1995); A. Fraser *et al.*, *Cell* 85:781-784 (1996); S. Nagata *et al.*, *Science* 267:1449-56 (1995)). Both are members of the TNF receptor family, which also include TNFR-2, low affinity NGFR, CD40, and CD30, among others (C.A. Smith *et al.*, *Science* 248: 1019-23 (1990); M. Tewari *et al.*, in *Modular Texts in Molecular and Cell Biology* M. Purton, Heldin, Carl, Ed. (Chapman and Hall, London, 1995). While family members are defined by the presence of cysteine-rich repeats in their extracellular domains, Fas/APO-1 and TNFR-1 also share a region of intracellular homology, appropriately designated the "death domain," which is distantly related to the *Drosophila* suicide gene, reaper (P. Golstein *et al.*, *Cell* 81:185-6 (1995); K. White *et al.*, *Science* 264:677-83 (1994)). This shared death domain suggests that both receptors interact with a related set of signal transducing molecules that, until recently, remained unidentified. Activation of Fas/APO-1 recruits the death domain-containing adapter molecule FADD/MORT1 (A.M. Chinnaiyan *et al.*, *Cell* 81:505-512 (1995); M. P. Boldin *et al.*, *J. Biol. Chem.* 270:7795-8 (1995); F.C. Kischkel *et al.*, *EMBO* 14:5579-5588 (1995)), which in turn binds and presumably activates FLICE/MACH1, a member of the ICE/CED-3 family of pro-apoptotic proteases (M. Muzio *et al.*, *Cell* 85: 817-827 (1996); M.P. Boldin *et al.*, *Cell* 85:803-815 (1996)). While the central role of Fas/APO-1 is to trigger cell death, TNFR-1 can signal an array of diverse biological activities-many of which stem from its ability to activate NF-kB (L.A. Tartaglia *et al.*, *Immunol Today* 13:151-153 (1992)). Accordingly, TNFR-1 recruits the multivalent adapter molecule TRADD, which like FADD, also contains a death domain (H. Hsu *et al.*, *Cell* 81:495-504 (1995); H. Hsu *et al.*, *Cell* 84:299-308 (1996)). Through its associations with a number of signaling molecules

including FADD, TRAF2, and RIP, TRADD can signal both apoptosis and NF-kB activation (H. Hsu *et al.*, *Cell* 84:299-308 (1996); H. Hsu *et al.*, *Immunity* 4:387-396 (1996)).

[0011] Recently, a new apoptosis inducing TNF ligand has been discovered. S.R. Wiley *et al.*, *Immunity* 3:673-682 (1995), named the new molecule, "TNF-related apoptosis-inducing ligand" or "TRAIL." R.M. Pitti *et al.*, *J. Biol. Chem.* 271:12687-12690 (1996), named the molecule "Apo-2 ligand" or "Apo-2L." This molecule was also disclosed in co-pending U.S. provisional patent application no. 60/013405. For convenience, this molecule will be referred to herein as TRAIL.

[0012] Unlike FAS ligand, whose transcripts appear to be largely restricted to stimulated T-cells, significant levels of TRAIL are detected in many human tissues (e.g., spleen, lung, prostate, thymus, ovary, small intestine, colon, peripheral blood lymphocytes, placenta, kidney), and it is constitutively transcribed by some cell lines. It has been shown that TRAIL acts independently from the FAS ligand (S.R. Wiley *et al.*, *supra*). It has also been shown that TRAIL activates apoptosis rapidly, within a time frame that is similar to death signaling by Fas/Apo-1L, but much faster than TNF-induced apoptosis. S.A. Marsters *et al.*, *Current Biology* 6:750-752 (1996). The inability of TRAIL to bind TNFR-1, Fas, or the recently identified DR3, suggests that TRAIL may interact with a unique receptor(s). Work to date suggests that there are several unique TNF receptors for TRAIL (see e.g., Pan *et al.*, *Science* 277:815-821 (1997)).

[0013] The effects of TNF family ligands and receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states.

SUMMARY OF THE INVENTION

[0014] The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding at least a portion of TR16 (e.g., a portion of a TR16-short or TR16-long polypeptide sequence). Thus, the present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the TR16-short receptor having the amino acid sequence shown in Figures 1A-E (in SEQ ID NO:2); or the TR16-long

receptor having the amino acid sequence shown in Figures 4A-E (SEQ ID NO:4); or the amino acid sequence encoded by the cDNA clone (HTWBD48 and/or HLICS62) deposited on August 12, 1999 as American Type Culture Collection ("ATCC") Deposit No. PTA-506. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209.

[0015] The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of TR16 polypeptides or peptides by recombinant techniques.

[0016] The invention further provides an isolated TR16 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

[0017] The present invention also provides diagnostic assays such as quantitative and diagnostic assays for detecting levels of TR16 protein. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of TR16, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors.

[0018] Tumor Necrosis Factor (TNF) family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cell proliferation, cytotoxicity, anti-viral activity, immunoregulatory activities, hematopoiesis, and the transcriptional regulation of several genes. Cellular responses to TNF-family ligands include not only normal physiological responses, but also diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis-programmed cell death is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes. Diseases associated with increased cell survival, unregulated cell proliferation, or the inhibition of apoptosis, include cancers, autoimmune disorders, viral infections, inflammation, graft vs. host disease, acute graft rejection, and chronic graft rejection. Diseases associated with increased apoptosis include AIDS, neurodegenerative disorders, myelodysplastic syndromes, ischemic injury, toxin-induced liver disease, septic shock, cachexia, and anorexia.

[0019] Thus, the invention further provides a method cells which express the TR16 polypeptide with a candidate compound and a TNF-family ligand (e.g. Neutrokin-alpha

or APRIL (*J. Exp. Med.* 188(6):1185-1190 (1998)), assaying a for inhibiting TR16 mediated signaling induced by a TNF-family ligand (e.g., Neutrokin-alpha (International Application Publication No. WO 98/18921)) which involves administering to a cell which expresses the TR16 polypeptide an effective amount of a TR16 antagonist capable of decreasing TR16 mediated signaling.

[0020] In a further aspect, the present invention is directed to a method for enhancing TR16 mediated signaling induced by a TNF-family ligand (e.g., Neutrokin-alpha) which involves administering to a cell which expresses the TR16 polypeptide an effective amount of a TR16 agonist capable of increasing TR16 mediated signaling.

[0021] Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit TR16 mediated signaling can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below (see, e.g., Examples 17 and 18). Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a TR16-mediated cellular response to a TNF-family ligand. The method involves contacting cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By the invention, a cell expressing the TR16 polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand.

BRIEF DESCRIPTION OF THE FIGURES

[0022] Figures 1A-E shows the nucleotide (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of the TR16-short receptor. Predicted amino acids 1 to 47 constitute the signal peptide (SEQ ID NO:2); amino acids 48 to 923 constitute the extracellular domain (SEQ ID NO:2); amino acids 924 to 948 constitute the transmembrane domain (SEQ ID NO:2); and amino acids 949 to 963 constitute the intracellular domain (SEQ ID NO:2).

[0023] Figure 2 shows the regions of similarity between the amino acid sequences of

the TR16-short receptor protein (SEQ ID NO:2), and the human TNFR 1 (SEQ ID NO:5), and OX40 (SEQ ID NO:6).

[0024] Figure 3 shows an analysis of the TR16-short amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. More specifically, Row I shows Garnier-Robson alpha-regions; Row II shows Chou-Fasman alpha-regions; Row III shows Garnier-Robson beta-regions; Row IV shows Chou-Fasman beta-regions; Row V shows Garnier-Robson turn-regions; Row VI shows Chou-Fasman turn-regions; Row VII shows Garnier-Robson coil-regions; Row VIII shows Kyte-Doolittle hydrophilic plot; Row IX shows Eisenberg alpha amphipathic regions; Row X shows Eisenberg beta-amphipathic regions; Row XI shows Karplus-Schulz flexible regions; Row XII shows Jameson-Wolf regions of high antigenic index; and Row XIII shows Emini surface-forming regions. In the "Antigenic Index - Jameson-Wolf" graph (Row XII), amino acid residues 51 to 67, 72 to 79, 94 to 104, 159 to 171, 180 to 185, 222 to 233, 238 to 242, 313 to 319, 325 to 346, 355 to 362, 385 to 395, 416 to 430, 456 to 465, 479 to 483, 530 to 535, 543 to 548, 569 to 579, 608 to 613, 627 to 639, 658 to 665, 702 to 707, 719 to 723, 744 to 747, 763 to 767, 837 to 842, 849 to 856, 886 to 893, and 950 to 955 in Figures 1A-E (SEQ ID NO:2) correspond to the shown highly antigenic regions of the TR16 protein. The information in each row is presented in tabular form in the column with the same Roman numeral in Table I, below.

[0025] Figures 4A-E show the nucleotide and deduced amino acid sequence of the TR16-long receptor. Predicted amino acids 1 to 47 constitute the signal peptide; amino acids 48 to 923 constitute the extracellular domain; amino acids 924 to 948 constitute the transmembrane domain; and amino acids 949 to 1027 constitute the intracellular domain.

[0026] Figure 5 shows an analysis of the TR16-long amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. More specifically, Row I shows Garnier-Robson alpha-regions; Row II shows Chou-Fasman alpha-regions; Row III shows Garnier-Robson beta-regions; Row IV shows Chou-Fasman beta-regions; Row V shows Garnier-Robson turn-regions; Row VI shows Chou-Fasman turn-regions; Row VII shows Garnier-Robson coil-regions; Row VIII shows Kyte-Doolittle hydrophilic plot; Row IX shows Eisenberg alpha amphipathic regions; Row X shows Eisenberg beta-amphipathic regions; Row XI shows Karplus-Schulz flexible regions; Row XII shows Jameson-Wolf regions of high

antigenic index; and Row XIII shows Emini surface-forming regions. The information in each row is presented in tabular form in the column with the same Roman numeral in Table II, below.

[0027] Figures 6A-B show an alignment between the amino acid sequence of TR16 short (SEQ ID NO:2 as shown in Figures 1A-E) and the amino acid sequence of SEC10 (SEQ ID NO:43) reported in Figure 10 of International Publication No. WO 00/78802. The alignment was carried out with Megalign using Clustal method with PAM250 weight table.

[0028] Figures 7A-B show an alignment between the amino acid sequence of TR16 long (SEQ ID NO:4 as shown in Figures 4A-E) and the amino acid sequence of SEC10 (SEQ ID NO:43) reported in Figure 10 of International Publication No. WO 00/78802. The alignment was carried out with Megalign using Clustal method with PAM250 weight table.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a TR16 polypeptide having the amino acid sequence shown in Figures 1A-E (SEQ ID NO:2) or shown in Figures 4A-E (SEQ ID NO:4). The TR16 polypeptides of the present invention share sequence homology with human TNFR1, and OX40 (Figure 2). Portions of the nucleotide sequence shown in Figures 1A-E (SEQ ID NO:1) were obtained by sequencing the cDNA clones HLICS62 and HTWBD48, which were deposited at the American Type Culture Collection, and given Accession Number PTA-506. The deposited HLICS62 clone is inserted in the pCMVSPORT 2.0 plasmid (Life Technologies, Rockville, MD) using the Sal I/Not I restriction endonuclease cleavage sites. The deposited HTWBD48 clone is inserted in the pSPORT1 plasmid (Life Technologies, Rockville, MD) using the Sal I/Not I restriction endonuclease cleavage sites.

NUCLEIC ACID MOLECULES

[0030] The determined nucleotide sequence of the TR16-short cDNA of Figures 1A-E (SEQ ID NO:1) contains an open reading frame encoding a protein of about 963 amino acid residues, with a predicted leader sequence of about 47 amino acid residues, and a

deduced molecular weight of about 106 kDa. The amino acid sequence of the predicted mature TR16-short receptor is shown in SEQ ID NO:2 from amino acid residue about 48 to residue about 963. Of the published members of the TNF receptor family, the TR16 polypeptides of the invention share the greatest degree of homology with human TNFR 1 (SEQ ID NO:5), and OX40 (SEQ ID NO:6) (See Figure 2), including significant sequence homology over multiple cysteine rich domains.

[0031] The determined nucleotide sequence of the TR16-long cDNA (Figures 4A-E (SEQ ID NO:3)) contains an open reading frame encoding a protein of about 1027 amino acid residues, with a predicted leader sequence of about 47 amino acid residues, and a deduced molecular weight of about 114 kDa. The amino acid sequence of the predicted mature TR16-long receptor is shown in Figures 4A-E (SEQ ID NO:4) from amino acid residue about 48 to residue about 1027.

[0032] To examine the tissue distribution of TR16, Northern blot analysis was performed. TR16 message was detected in multiple human tissues at varying levels of expression, including, brain, spleen, and testis.

[0033] As indicated, the present invention also provides the mature form(s) of the TR16 receptors of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

[0034] The present invention provides a nucleotide sequence encoding the mature TR16-long polypeptide having the amino acid sequence shown in Figures 4A-E (SEQ ID NO:4). By the mature TR16-long protein having the amino acid sequence shown in Figures 4A-E is meant the mature form(s) of the TR16-long receptor predicted by computer analysis or produced by expression of the coding sequence shown in Figures 4A-E in a mammalian cell (e.g., COS cells, as described below). As indicated below, the mature TR16-long receptor having the amino acid sequence encoded by the coding

sequence shown in Figures 4A-E, may or may not differ from the predicted mature TR16-long protein shown in Figures 4A-E (SEQ ID NO:4, amino acids from about 48 to about 1027) depending on the accuracy of the predicted cleavage site based on computer analysis.

[0035] The present invention further provides a nucleotide sequence encoding the mature TR16-short polypeptide having the amino acid sequence shown in Figures 1A-E (SEQ ID NO:2). By the mature TR16-short protein having the amino acid sequence encode shown in Figures 1A-E is meant the mature form(s) of the TR16-short receptor predicted by computer analysis or produced by expression of the coding sequence shown in Figures 1A-E in a mammalian cell (e.g., COS cells, as described below). As indicated below, the mature TR16-short receptor having the amino acid sequence encoded by the coding sequence shown in Figures 1A-E, may or may not differ from the predicted mature TR16-short protein shown in SEQ ID NO:2 (amino acids from about 48 to about 963) depending on the accuracy of the predicted cleavage site based on computer analysis.

[0036] Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the method of McGeoch (*Virus Res.* 3:271-286 (1985)) and von Heinje (*Nucleic Acids Res.* 14:4683-4690 (1986)) can be used. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. von Heinje, *supra*. However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

[0037] In the present case, the predicted amino acid sequence of the complete TR16 polypeptides of the present invention was analyzed by a computer program ("PSORT"). See K. Nakai and M. Kanehisa, *Genomics* 14:897-911 (1992). PSORT is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage site between amino acids 47 and 48 of the TR16 polypeptide sequence in Figures 1A-E (SEQ ID NO:2) and Figures 4A-E (SEQ ID NO:4). Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying a simple form of the (-1, -3) rule of von Heinje. von Heinje, *supra*. Thus, the leader sequence for both of the TR16-short and TR16-long proteins are predicted to consist of amino acid residues from about 1

to about 47 in Figures 1A-E and Figures 4A-E, respectively, while the mature TR16-short protein is predicted to consist of residues from about 48 to 963 in Figures 1A-E (SEQ ID NO:2), and the mature TR16-long protein is predicted to consist of residues from about 48 to 1027 in Figures 4A-E (SEQ ID NO:4).

[0038] As one of ordinary skill would appreciate, due to the possibilities of sequencing errors, as well as the variability of cleavage sites for leaders in different known proteins, the predicted TR16-short polypeptide, a portion of which is encoded by the deposited cDNA, comprises about 963 amino acids, but may be anywhere in the range of 953-973 amino acids; and the predicted leader sequence of this protein is about 47 amino acids, but may be anywhere in the range of about 37 to about 57 amino acids. Similarly, the predicted TR16-long polypeptide, comprises about 1027 amino acids, but may be anywhere in the range of 1017 to about 1037 amino acids; and the predicted leader sequence of this protein is about 47 amino acids, but may be anywhere in the range of about 37 to about 57 amino acids. It will further be appreciated that, the domains described herein have been predicted by computer analysis, and accordingly, that depending on the analytical criteria used for identifying various functional domains, the exact "address" of, for example, the extracellular domain, intracellular domain, cysteine-rich motifs, and transmembrane domain of TR16 may differ slightly. For example, the exact location of the TR16 extracellular domain in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4) may vary slightly (e.g., the address may "shift" by about 1 to about 20 residues, more likely about 1 to about 5 residues) depending on the criteria used to define the domain. In any event, as discussed further below, the invention further provides polypeptides having various residues deleted from the N-terminus and/or C-terminus of the complete TR16, including polypeptides lacking one or more amino acids from the N-termini of the TR16 extracellular domains described herein, which constitute soluble forms of the extracellular domain of the TR16 polypeptides respectively.

[0039] In specific embodiments, the present invention encompasses polypeptides comprising amino acid sequence that are not contained in the amino acid sequence of SEC10 (SEQ ID NO:43) reported in Figure 10 of International Publication No. WO 00/78802, as well as polynucleotides encoding such amino acid sequences and antibodies and antibody fragments specifically binding to such amino acid sequences. Such

polypeptide sequences are depicted, for example, as non-shaded regions in Figures 6A-B and 7A-B. For example, the present invention encompasses polypeptides comprising the amino acid sequence and fragments thereof (which fragments have lengths as defined below, for example) from Met-1 (M-1) to Glu 114 (E-114) or from Ala-566 (A-566) to Asn-963 (N-963) of the TR16 short polypeptide as shown in Figures 1A-E, and from Met-1 (M-1) to Glu 114 (E-114) or from Ala-566 (A-566) to Ile 1029 (I-1027) of the TR16 long polypeptide, as shown in Figures 4A-E. In another specific embodiment, the invention encompasses polypeptides comprising the amino acid sequence from Met-115 (M-115) to Trp-565 (W-565) of the TR16 short or TR16 long polypeptides of Figures 1A-E or 4A-E, respectively. In additional specific embodiments, the polypeptides of the present invention, including those with at least 80%, 85%, 90% identity, and more preferably at least 95%, 96%, 97%, 98%, or 99% identity to either the amino acid sequence of the TR16 short or TR16 long polypeptides (Figures 1A-E or 4A-E, respectively) or to the amino acid sequence from Met-115 (M-115) to Trp-565 (W-565) of the TR16 short or TR16 long polypeptides contain only one or neither of the Glu (E) residues corresponding to those present at positions 220 and 221 of SEC10 (SEQ ID NO:43) reported in Figure 10 of International Publication No. WO 00/78802. In addition, the invention encompasses polynucleotides encoding these amino acid sequences and fragments thereof (which fragments have lengths as defined below, for example), as well as nucleotide sequences with at least 80%, 85%, 90% identical, and more preferably at least 95%, 96%, 97%, 98%, or 99% identity to such encoding polynucleotides, in addition to antibodies and antibody fragments specifically binding to such amino acid sequences.

[0040] As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

[0041] By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include

recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically. However, a nucleic acid molecule contained in a clone that is a member of a mixed clone library (e.g., a genomic or cDNA library) and that has not been isolated from other clones of the library (e.g., in the form of a homogeneous solution containing the clone without other members of the library) or a chromosome isolated or removed from a cell or a cell lysate (e.g., a "chromosome spread", as in a karyotype), is not "isolated" for the purposes of this invention.

[0042] Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in Figures 1A-E (SEQ ID NO:1); DNA molecules comprising the coding sequence for the complete (full-length) and/or mature TR16-short protein shown in Figures 1A-E (SEQ ID NO:2); and DNA molecules which comprise a sequence substantially different from those described above, but which, due to the degeneracy of the genetic code, still encode the TR16-short protein. Additionally, isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in Figures 4A-E (SEQ ID NO:3); DNA molecules comprising the coding sequence for the complete (full-length) and/or mature TR16-long protein shown in Figures 4A-E (SEQ ID NO:4) and DNA molecules which comprise a sequence substantially different from those described above, but which, due to the degeneracy of the genetic code, still encode the TR16-long protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

[0043] In addition, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of Figures 1A-E (SEQ ID NO:1) and Figures 4A-E (SEQ ID NO:3) which have been determined in part from the following related cDNA clones: HTWBD48 and HLICS62.

[0044] In another aspect, the invention provides isolated nucleic acid molecules having a polynucleotide sequence encoding the TR16 polypeptide having an amino acid sequence as encoded by a cDNA clone contained in the plasmids deposited as ATCC Deposit No. PTA-506. The invention further provides an isolated nucleic acid molecule

having the nucleotide sequence shown in Figures 1A-E (SEQ ID NO:1), or a nucleic acid molecule having the nucleotide sequence shown in Figures 4A-E (SEQ ID NO:3), or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful, for example, as probes for gene mapping by *in situ* hybridization with chromosomes, and for detecting expression of the TR16 gene in human tissue, for instance, by Taqman® or Northern blot analysis.

[0045] The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of deposited cDNA or the nucleotide sequence shown in Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3) is intended DNA fragments at least about 15nt, and more preferably at least about 20 nt, at least about 24 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 100 nt, at least about 150 nt, at least about 200 nt, at least about 250 nt, at least about 300 nt in length which are useful, for example, as diagnostic probes and primers as discussed herein. Of course, larger fragments 350-1500 nt in length are also useful according to the present invention, as are fragments corresponding to most, if not all, of the DNA sequence of one or more of the deposited cDNA plasmids, or as shown in Figures 1A-E (SEQ ID NO:1), or the complementary strand thereto, or as shown in Figures 4A-E (SEQ ID NO:3), or the complementary strand thereto. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of a deposited cDNA, or the nucleotide sequence as shown in Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3). In this context "about" includes the particularly recited size, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. In specific embodiments, the fragments of the invention comprise, or alternatively consist of, nucleotides 178 to 198, 298 to 321, 496 to 519, 643 to 666, 730 to 753, 838 to 861, 988 to 1011, 1072 to 1095, 1252 to 1275, 1381 to 1404, 1474 to 1497, 1576 to 1599, 1714 to 1737, 1978 to 2001, 2152 to 2175, 2341 to 2364, 2440 to 2463, 2539 to 2562, 2668 to 2691, and/or 2848 to 2871 of Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3), or the complementary strand thereto, or the cDNA contained in a deposited plasmid. In further specific embodiments, the fragments of the invention comprise, or alternatively consist of, nucleotides 2848 to 3012 and/or 3013 to 3036 of Figures 4A-E (SEQ ID NO:3), or the

complementary strand thereto.

[0046] In further specific embodiments, the fragments of the invention comprise, or alternatively consist of, nucleotides 500 to 1330, and/or 2500 to 2884 of Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3), or the complementary strand thereto.

[0047] Representative examples of TR16-short and/or TR16-long polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to 33, 34 to 66, 67 to 99, 100 to 141, 142 to 174, 175 to 207, 208 to 243, 244 to 288, 289 to 321, 322 to 354, 355 to 390, 391 to 423, 424 to 480, 481 to 513, 514 to 546, 547 to 579, 580 to 621, 622 to 660, 661 to 708, 709 to 750, 751 to 810, 811 to 868, 869 to 990, 991 to 1032, 1033 to 1065, 1066 to 1140, 1141 to 1200, 1201 to 1242, 1243 to 1278, 1279 to 1350, 1351 to 1401, 1402 to 1452, 1453 to 1509, 1510 to 1560, 1561 to 1629, 1630 to 1689, 1690 to 1749, 1750 to 1803, 1804 to 1869, 1870 to 1941, 1942 to 2016, 2017 to 2070, 2071 to 2130, 2131 to 2190, 2191 to 2250, 2251 to 2310, 2311 to 2400, 2401 to 2472, 2473 to 2580, 2581 to 2640, 2641 to 2700, 2701 to 2757, 2758 to 2770, and/or 2771 to 2844, of Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3), or the complementary strand thereto. Additional representative examples of TR16-short polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 2845 to 2889, 2890 to 3060, 3061 to 3120, 3121 to 3180, 3181 to 3240, 3421 to 3300, and/or 3301 to 3390 of Figures 1A-E (SEQ ID NO:1) or the complementary strand thereto. Additional representative examples of TR16-long polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 2845 to 2940, 2941 to 3000, 3001 to 3081, 3082 to 3181, 3182 to 3300, 3301 to 3420, and/or 3421 to 3556 of Figures 4A-E (SEQ ID NO:3); or the complementary strand thereto, or the cDNA contained in one of the deposited cDNA clones. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

[0048] In specific embodiments, the polynucleotide fragments of the invention comprise, or alternatively, consist of, a sequence from nucleotide 868 to 1032, 1066 to 1278, 1804 to 2016, and/or 2473 to 2757 of Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3), or the complementary strand thereto. Polypeptides encoded by these polynucleotide fragments are also encompassed by the invention.

[0049] Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a TR16 functional activity. By a polypeptide demonstrating a TR16 "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) TR16 protein (e.g., TR16 long or short protein). Such functional activities include, but are not limited to, biological activity, antigenicity (ability to bind (or compete with a TR16 polypeptide for binding) to an anti-TR16 antibody), immunogenicity (ability to generate antibody which binds to a TR16 polypeptide), ability to form multimers with TR16 polypeptides of the invention, and ability to bind to a receptor or ligand for a TR16 polypeptide (e.g., Neutrokin- α).

[0050] The functional activity of TR16 polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

[0051] For example, in one embodiment where one is assaying for the ability to bind or compete with full-length TR16 polypeptides for binding to anti-TR16 antibody various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

[0052] In another embodiment, where a TR16 ligand is identified (e.g., Neutrokin- α), or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., *et al.*, *Microbiol. Rev.* 59:94-123 (1995). In another embodiment, physiological correlates of TR16 binding to its

substrates (signal transduction) can be assayed.

[0053] In addition, assays described herein and otherwise known in the art may routinely be applied to measure the ability of TR16 polypeptides and fragments, variants derivatives and analogs thereof to elicit TR16 related biological activity. For example, techniques described herein (see e.g., Examples 16, 17 and 18) and otherwise known in the art may be applied or routinely modified to assay for the ability of the compositions of the invention to inhibit or stimulate B cell proliferation (e.g., Neutrokin-alpha mediated B cell proliferation).

[0054] Other methods will be known to the skilled artisan and are within the scope of the invention.

[0055] Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding a member selected from the group: a polypeptide comprising or alternatively, consisting of, the TR16 receptor extracellular domain (amino acid residues from about 48 to about 923 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4)); a polypeptide comprising, or alternatively consisting of, the TR16 cysteine rich domain (amino acid residues from about 289 to about 920 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4)) or one or more TR16 cysteine rich motifs amino acid residues from about 290 to 344, 356 to 426, 602 to 672, and/or 825 to 919 of Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of the TR16-long transmembrane domain (amino acid residues from about 924 to about 948 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4)); a polypeptide comprising, or alternatively consisting of, the TR16-short intracellular domain (amino acid residues from about 949 to about 963 in Figures 1A-E (SEQ ID NO:2)); and/or a polypeptide comprising, or alternatively consisting of, the TR16-long intracellular domain (amino acid residues from about 949 to about 1027 in Figures 4A-E (SEQ ID NO:4)). Since the location of these domains have been predicted by computer analysis, one of ordinary skill would appreciate that the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to 15 amino acid residues) depending on the criteria used to define each domain.

[0056] Preferred nucleic acid fragments of the invention encode a full-length TR16 polypeptide lacking the nucleotides encoding the amino terminal methionine in Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3), as it is known that the methionine

is cleaved naturally and such sequences may be useful in genetically engineering TR16 expression vectors. Polypeptides encoded by such nucleic acids are also encompassed by the invention.

[0057] Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the TR16 receptor proteins. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising, or alternatively consisting of, amino acid residues from about 51 to about 67 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 72 to about 79 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 94 to about 104 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 159 to about 171 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 180 to about 185 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 222 to about 233 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 238 to about 242 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 313 to about 319 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 325 to about 348 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 355 to about 362 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 385 to about 395 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 418 to about 430 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 456 to about 465 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a

polypeptide comprising, or alternatively consisting of, amino acid residues from about 479 to about 483 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 530 to about 535 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 543 to about 548 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 569 to about 579 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 608 to about 615 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 627 to about 639 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 658 to about 665 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 702 to about 707 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 719 to about 724 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 744 to about 747 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 763 to about 767 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 837 to about 842 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 849 to about 856 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 886 to about 813 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); and a polypeptide comprising, or alternatively consisting of, amino acid residues from about 950 to about 955 in Figures 1A-E (SEQ ID NO:2). In this context the inventors have determined that the above polypeptide fragments are antigenic regions of the TR16 proteins. Methods for determining other such epitope-bearing portions of the TR16

proteins are described in detail below. Polypeptides encoded by these nucleic acids are also encompassed by the invention.

[0058] It is believed that the extracellular cysteine rich motifs of TR16 disclosed in Figures 1A-E and Figures 4A-E are important for interactions between TR16 and its ligands. Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of amino acid residues 290 to 344, 356 to 426, 602 to 672, and/or 825 to 919 of Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4). In a specific embodiment the polynucleotides encoding TR16 polypeptides of the invention comprise, or alternatively consist of any combination of one, two, three or all four of the extracellular cysteine rich motifs disclosed in Figures 1A-E or Figures 4A-E. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0059] In additional embodiments, the polynucleotides of the invention encode functional attributes of TR16. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of TR16.

[0060] The data representing the structural or functional attributes of TR16-short (Figure 3 and/or Table I) and TR16-long (Figure 5 and/or table II), as described above, was generated using the various modules and algorithms of the DNA*STAR set on default parameters. The data presented in columns VIII, XII, and XIII of Tables I and II can be used to determine regions of TR16-short and TR16-long which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, XII, and/or XIII by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

[0061] Certain preferred regions in these regards are set out in Figures 3 or 5, but may, as shown in Table I or Table II, respectively, be represented or identified by using tabular

representations of the data presented in Figures 3 or 5, respectively. The DNA*STAR computer algorithm used to generate Figures 3 and 5 (set on the original default parameters) was used to present the data in Figures 3 and 5 in a tabular format (See Table I and II, respectively). The tabular format of the data in Figures 3 and 5 may be used to easily determine specific boundaries of a preferred region.

[0062] The above-mentioned preferred regions set out in Figures 3 and 5 and in Table I and II, include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequences set out in Figures 1A-E and Figures 4A-E, respectively. As set out in Figures 3 and 5 and in Tables I and II, such preferred regions include Garnier-Robson alpha-regions (column I), beta-regions (column III), turn-regions (column V), and coil-regions (column VII), Chou-Fasman alpha-regions (column II), beta-regions (column IV), and turn-regions (column VI), Kyte-Doolittle hydrophilic regions (column VIII), Eisenberg alpha- (column IX) and beta-amphipathic regions (column X), Karplus-Schulz flexible regions (column XI), Jameson-Wolf regions of high antigenic index (column XII) and Emini surface-forming regions (column XIII).

Table I

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Met	1	.	A	B	-0.64	.	*	.	-0.30	0.52
Leu	2	.	A	B	-0.14	.	*	.	-0.60	0.41
Phe	3	.	A	B	-0.10	.	*	.	-0.30	0.63
Arg	4	.	A	B	0.08	.	*	.	-0.30	0.63
Ala	5	T	T	.	-0.39	*	*	F	1.40	1.18
Arg	6	.	.	B	.	.	T	.	0.32	*	*	F	1.00	1.01
Gly	7	T	C	0.79	.	*	F	1.50	1.01
Pro	8	T	C	1.60	.	*	F	1.35	0.99
Val	9	T	.	.	1.14	.	*	F	1.66	0.99
Arg	10	.	.	B	1.44	*	*	F	1.57	0.99
Gly	11	T	T	.	0.99	*	*	F	2.18	0.67
Arg	12	T	T	.	1.44	*	*	F	2.49	0.90
Gly	13	T	T	.	1.44	*	*	F	3.10	0.90
Trp	14	T	T	.	1.71	*	*	F	2.64	1.40
Gly	15	C	1.60	*	*	F	1.78	0.72
Arg	16	.	A	C	1.36	*	*	F	1.42	1.26
Pro	17	.	A	C	1.03	*	*	F	1.45	1.21
Ala	18	.	A	C	1.49	*	.	F	1.78	1.90
Glu	19	.	A	B	1.89	*	*	F	1.92	1.90
Ala	20	.	A	B	1.89	*	*	F	2.26	2.40
Pro	21	T	T	.	1.89	*	*	F	3.40	2.35
Arg	22	T	T	.	1.80	*	*	F	3.06	2.66
Arg	23	T	T	.	2.18	*	*	F	2.72	3.53
Gly	24	T	T	.	1.97	*	*	F	2.66	3.53
Arg	25	T	.	.	2.27	*	*	F	2.40	2.79
Ser	26	T	C	2.18	*	.	F	2.04	1.50
Pro	27	T	C	1.86	*	.	F	2.32	2.03
Pro	28	T	T	.	1.16	.	*	F	2.80	1.60
Trp	29	T	T	.	1.21	.	.	F	1.62	1.21
Ser	30	T	C	0.21	.	.	F	0.99	0.82
Pro	31	T	T	.	-0.16	.	.	.	0.76	0.37
Ala	32	T	T	.	-0.61	.	.	.	0.48	0.19
Trp	33	.	.	B	.	.	T	.	-0.69	.	.	.	-0.20	0.08
Ile	34	.	.	B	-0.99	.	.	.	-0.40	0.05
Cys	35	.	A	B	-1.50	.	.	.	-0.60	0.05
Cys	36	.	A	B	-1.88	.	.	.	-0.60	0.04
Trp	37	.	A	B	-1.63	.	.	.	-0.60	0.06
Ala	38	.	A	B	-2.01	.	.	.	-0.60	0.11
Leu	39	.	A	.	.	T	.	.	-1.12	.	.	.	-0.20	0.11
Ala	40	.	A	.	.	T	.	.	-1.04	.	.	.	-0.20	0.18
Gly	41	.	A	.	.	T	.	.	-0.97	.	.	.	-0.20	0.18
Cys	42	.	A	.	.	T	.	.	-0.97	.	.	.	-0.20	0.22
Gln	43	.	A	B	-0.97	.	.	.	-0.60	0.23
Ala	44	.	A	B	-0.50	.	.	.	-0.60	0.23
Ala	45	.	A	B	0.09	.	*	.	-0.60	0.43
Trp	46	.	.	B	.	.	T	.	-0.38	.	*	.	0.10	0.41
Ala	47	.	.	B	.	.	T	.	0.08	.	*	.	-0.20	0.34
Gly	48	.	.	B	.	.	T	.	-0.22	.	*	.	-0.20	0.51
Asp	49	T	C	0.07	.	.	F	0.45	0.65

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Leu	50	T	C	0.36	.	.	F	1.39	0.87
Pro	51	T	C	0.34	*	*	F	1.88	1.17
Ser	52	T	T	.	1.04	*	.	F	2.27	0.94
Ser	53	T	T	.	1.18	*	.	F	2.76	2.24
Ser	54	T	T	.	0.37	*	.	F	3.40	2.24
Ser	55	T	T	.	0.97	*	.	F	2.76	1.38
Arg	56	T	C	0.97	*	.	F	2.22	1.59
Pro	57	T	T	.	0.60	*	.	F	2.08	1.83
Leu	58	C	0.90	*	.	F	1.19	0.73
Pro	59	T	C	1.20	*	*	F	1.05	0.65
Pro	60	T	T	.	1.54	*	.	F	1.25	0.73
Cys	61	T	T	.	1.43	*	.	F	1.70	1.76
Gln	62	.	.	B	.	.	T	.	1.40	.	.	F	1.90	1.90
Glu	63	.	.	B	2.18	.	.	F	2.00	1.93
Lys	64	.	.	B	1.69	.	.	F	2.30	4.90
Asp	65	T	.	.	1.90	.	*	F	3.00	2.45
Tyr	66	T	.	.	2.32	.	*	.	2.55	2.45
His	67	.	.	B	2.01	.	.	.	1.55	1.92
Phe	68	.	.	B	2.01	.	.	.	0.65	1.66
Glu	69	.	.	B	1.30	.	.	.	0.35	1.83
Tyr	70	.	.	B	1.30	.	*	.	-0.10	0.72
Thr	71	T	.	.	1.24	.	.	.	1.05	1.39
Glu	72	T	.	.	0.98	.	.	F	1.84	1.08
Cys	73	T	.	.	1.33	.	.	F	1.73	0.92
Asp	74	T	T	.	1.03	*	.	F	2.57	0.63
Ser	75	T	T	.	1.39	*	.	F	2.91	0.49
Ser	76	T	T	.	1.41	*	*	F	3.40	1.79
Gly	77	T	T	.	1.52	.	*	F	2.76	1.12
Ser	78	.	.	.	B	T	.	.	1.33	.	*	F	2.02	1.64
Arg	79	.	.	.	B	T	.	.	0.74	*	*	F	1.53	0.91
Trp	80	.	.	B	B	.	.	.	0.16	.	*	.	0.64	0.93
Arg	81	.	.	B	B	.	.	.	0.24	*	*	.	-0.30	0.49
Val	82	.	.	B	B	.	.	.	0.59	*	*	.	-0.30	0.38
Ala	83	.	.	B	B	.	.	.	0.59	*	*	.	-0.60	0.59
Ile	84	.	.	B	.	.	T	.	-0.11	.	*	.	0.10	0.40
Pro	85	T	C	-0.68	.	*	F	0.15	0.55
Asn	86	T	T	.	-0.79	.	*	F	0.35	0.40
Ser	87	.	.	B	.	T	T	.	-0.60	.	.	F	0.65	0.96
Ala	88	.	.	B	-0.31	.	.	.	0.50	0.33
Val	89	.	.	B	0.23	.	.	.	0.50	0.28
Asp	90	.	.	B	-0.37	.	.	.	0.50	0.20
Cys	91	.	.	B	.	.	T	.	-0.58	.	.	.	0.10	0.17
Ser	92	.	.	B	.	.	T	.	-0.28	.	.	F	0.25	0.35
Gly	93	.	.	B	.	.	T	.	0.10	.	*	F	0.85	0.35
Leu	94	T	C	0.10	*	*	F	1.54	1.00
Pro	95	C	0.21	*	*	F	0.93	0.55
Asp	96	T	C	0.53	*	*	F	2.22	1.10
Pro	97	.	.	B	.	.	T	.	0.88	*	*	F	2.36	1.32
Val	98	T	T	.	1.22	*	*	F	3.40	1.70

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Arg	99	.	.	B	.	.	T	.	1.37	*	*	F	2.66	1.77
Gly	100	T	T	.	1.27	*	*	F	2.57	0.61
Lys	101	T	T	.	0.57	*	*	F	2.38	1.19
Glu	102	.	.	B	.	.	T	.	0.48	.	*	F	1.49	0.53
Cys	103	.	.	B	.	.	T	.	0.67	*	*	.	0.70	0.71
Thr	104	.	.	B	-0.03	*	*	.	0.50	0.19
Phe	105	.	.	B	0.01	.	.	.	-0.10	0.11
Ser	106	.	.	B	-0.38	.	.	.	-0.40	0.28
Cys	107	T	T	.	-0.38	.	.	.	0.20	0.19
Ala	108	T	T	.	0.04	.	.	.	0.50	0.38
Ser	109	T	C	-0.46	.	.	F	0.45	0.45
Gly	110	T	C	0.24	.	.	F	0.45	0.69
Glu	111	.	A	C	-0.06	.	*	F	0.80	1.18
Tyr	112	A	A	0.66	.	.	.	0.30	0.87
Leu	113	A	A	1.24	.	*	.	0.75	1.76
Glu	114	A	A	1.54	.	*	.	0.75	1.63
Met	115	A	A	1.03	.	*	.	0.45	1.80
Lys	116	.	A	.	.	T	.	.	0.37	.	*	F	1.00	1.62
Asn	117	.	A	.	.	T	.	.	0.31	.	*	F	0.85	0.50
Gln	118	.	A	.	.	T	.	.	1.17	.	*	F	0.25	0.68
Val	119	.	A	B	0.50	.	*	.	0.60	0.68
Cys	120	.	A	B	0.76	.	*	.	0.61	0.23
Ser	121	.	.	B	.	.	T	.	0.71	.	*	.	0.72	0.13
Lys	122	.	.	B	.	.	T	.	0.37	*	.	F	1.78	0.30
Cys	123	.	.	B	.	.	T	.	0.06	*	.	F	2.39	0.56
Gly	124	T	T	.	0.67	*	.	F	3.10	0.60
Glu	125	T	.	.	1.03	*	.	F	2.29	0.47
Gly	126	.	.	B	.	.	T	.	0.52	*	.	F	1.33	1.18
Thr	127	.	.	B	.	.	T	.	0.13	*	.	F	0.87	0.98
Tyr	128	.	.	B	.	.	T	.	0.50	.	*	F	0.56	0.56
Ser	129	.	.	B	.	.	T	.	0.50	*	*	.	-0.20	0.76
Leu	130	.	.	B	-0.39	*	*	F	-0.25	0.52
Gly	131	T	T	.	0.00	*	*	F	0.35	0.23
Ser	132	T	C	-0.39	.	*	F	0.45	0.35
Gly	133	T	C	-0.14	.	*	F	0.15	0.37
Ile	134	T	C	0.16	.	*	F	1.05	0.62
Lys	135	.	A	B	0.68	.	*	F	0.75	0.80
Phe	136	.	A	B	1.02	.	.	F	0.45	0.85
Asp	137	.	A	B	1.32	.	*	F	0.90	2.02
Glu	138	.	A	B	0.86	.	*	F	0.90	1.75
Trp	139	.	A	.	.	T	.	.	1.53	.	*	F	1.30	1.66
Asp	140	.	A	C	0.90	.	*	F	1.10	1.54
Glu	141	.	A	C	1.26	*	.	F	0.65	0.90
Leu	142	T	C	0.56	*	.	.	0.90	0.85
Pro	143	T	C	0.26	*	.	.	0.90	0.44
Ala	144	T	T	.	0.54	*	.	.	0.50	0.34
Gly	145	T	C	-0.34	*	.	.	0.00	0.66
Phe	146	C	-0.93	*	.	.	-0.20	0.30
Ser	147	C	-0.43	*	.	.	-0.20	0.30

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Asn	148	.	.	B	B	.	.	.	-0.92	*	.	.	-0.60	0.44
Ile	149	.	.	B	B	.	.	.	-0.93	*	.	.	-0.60	0.44
Ala	150	.	.	B	B	.	.	.	-0.59	*	.	.	-0.60	0.32
Thr	151	.	.	B	B	.	.	.	-0.20	*	.	.	-0.60	0.34
Phe	152	.	.	B	B	.	.	.	-0.76	*	.	.	-0.60	0.69
Met	153	.	.	B	B	.	.	.	-1.61	*	.	.	-0.60	0.51
Asp	154	.	.	B	B	.	.	.	-1.07	*	.	.	-0.60	0.26
Thr	155	.	.	B	B	.	.	.	-0.69	.	.	.	-0.60	0.30
Val	156	.	.	B	B	.	.	.	-0.68	*	.	.	0.04	0.47
Val	157	.	.	B	B	.	.	.	0.02	*	.	F	0.53	0.37
Gly	158	T	C	0.32	.	*	F	1.47	0.43
Pro	159	T	T	.	0.43	.	*	F	2.61	0.78
Ser	160	T	T	.	0.53	.	*	F	3.40	2.06
Asp	161	T	T	.	1.39	.	*	F	3.06	3.22
Ser	162	.	.	B	1.90	.	*	F	2.43	3.48
Arg	163	.	.	B	.	.	T	.	1.58	.	*	F	2.60	2.57
Pro	164	T	T	.	1.79	.	*	F	2.82	0.82
Asp	165	T	T	.	2.09	.	*	F	2.79	0.99
Gly	166	T	T	.	1.79	.	*	F	3.10	0.81
Cys	167	T	.	.	1.79	.	*	F	2.29	0.70
Asn	168	T	.	.	1.39	.	*	F	1.98	0.56
Asn	169	T	T	.	0.71	.	.	F	0.97	0.60
Ser	170	T	T	.	0.50	*	.	F	0.66	0.78
Ser	171	T	T	.	0.96	*	*	F	0.35	0.75
Trp	172	.	.	B	.	.	T	.	1.28	*	*	F	0.25	0.92
Ile	173	.	.	B	.	.	T	.	1.28	*	*	F	0.25	0.68
Pro	174	.	.	B	.	.	T	.	1.03	.	*	F	0.25	0.81
Arg	175	T	T	.	0.44	.	.	F	0.50	1.21
Gly	176	T	C	0.74	.	.	F	0.60	1.21
Asn	177	C	0.73	*	.	F	1.00	1.36
Tyr	178	C	1.62	.	.	.	1.04	0.93
Ile	179	.	.	B	1.94	.	*	F	0.88	1.51
Glu	180	.	.	B	1.83	.	*	F	1.82	1.84
Ser	181	.	.	B	2.18	.	.	F	2.46	1.96
Asn	182	T	T	.	1.51	.	.	F	3.40	4.67
Arg	183	T	T	.	1.44	.	*	F	3.06	1.45
Asp	184	T	T	.	1.48	.	*	F	2.72	1.56
Asp	185	T	T	.	1.18	.	*	F	2.23	0.72
Cys	186	.	.	B	B	.	.	.	0.67	.	*	.	0.94	0.49
Thr	187	.	.	B	B	.	.	.	-0.22	.	*	.	0.30	0.24
Val	188	.	.	B	B	.	.	.	-0.58	.	*	.	-0.60	0.10
Ser	189	.	.	B	B	.	.	.	-1.17	*	.	.	-0.60	0.30
Leu	190	.	.	B	B	.	.	.	-2.02	*	*	.	-0.60	0.21
Ile	191	.	.	B	B	.	.	.	-1.39	.	*	.	-0.60	0.21
Tyr	192	.	.	B	B	.	.	.	-1.89	.	*	.	-0.60	0.21
Ala	193	.	.	B	B	.	.	.	-0.99	.	*	.	-0.60	0.21
Val	194	.	.	B	B	.	.	.	-0.64	.	.	.	-0.32	0.60
His	195	.	.	B	B	.	.	.	-0.13	.	.	.	0.26	0.77
Leu	196	.	.	B	B	.	.	.	0.41	.	.	.	1.29	1.02

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

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Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
His	246	.	.	B	.	.	T	.	-0.03	.	*	.	-0.20	0.41
Ser	247	.	.	B	.	.	T	.	0.01	.	*	.	-0.20	0.34
Val	248	.	.	B	0.00	.	.	.	-0.10	0.51
Met	249	.	.	B	0.00	.	*	.	-0.10	0.50
Leu	250	.	.	B	.	.	T	.	-0.01	.	*	.	0.10	0.37
Lys	251	.	.	B	.	.	T	.	0.02	*	*	F	0.25	0.72
Ser	252	T	C	-0.57	*	*	F	0.60	1.16
Gly	253	T	C	-0.52	*	*	F	0.45	0.99
Thr	254	.	.	B	B	.	.	.	-0.17	*	.	F	-0.15	0.41
Asn	255	.	.	B	B	.	.	.	0.36	.	*	F	-0.45	0.48
Ile	256	.	.	B	B	.	.	.	0.42	.	*	.	-0.60	0.51
Leu	257	.	.	B	B	.	.	.	0.41	.	*	.	-0.60	0.69
Tyr	258	.	.	B	B	.	.	.	0.44	.	*	.	-0.60	0.62
Trp	259	.	.	B	B	.	.	.	0.41	.	.	.	-0.45	1.27
Arg	260	.	.	B	B	.	.	.	-0.48	.	*	.	-0.45	1.52
Thr	261	.	.	B	B	.	.	.	-0.40	.	*	F	-0.45	0.68
Thr	262	.	.	B	B	.	.	.	-0.19	.	*	F	-0.45	0.53
Gly	263	.	.	B	B	.	.	.	-0.29	.	*	.	-0.30	0.27
Ile	264	.	.	B	B	.	.	.	-0.30	.	*	.	-0.60	0.19
Leu	265	.	.	B	.	.	T	.	-0.37	.	*	.	-0.20	0.17
Met	266	.	.	B	.	.	T	.	-0.64	*	.	.	0.10	0.35
Gly	267	.	.	B	.	.	T	.	-1.19	*	.	F	0.34	0.50
Ser	268	.	.	B	.	.	T	.	-0.80	*	.	F	0.43	0.45
Lys	269	.	.	B	-0.12	*	.	F	0.92	0.91
Ala	270	.	.	B	-0.17	*	.	F	1.46	1.42
Val	271	.	.	B	B	.	.	.	-0.38	*	.	F	0.90	0.79
Lys	272	.	.	B	B	.	.	.	-0.89	*	.	F	0.81	0.33
Pro	273	.	.	B	B	.	.	.	-0.54	*	.	.	-0.33	0.24
Val	274	.	.	B	B	.	.	.	-0.59	*	.	.	-0.12	0.64
Leu	275	.	.	B	B	.	.	.	-0.89	.	.	.	0.39	0.52
Val	276	.	.	B	B	.	.	.	-0.34	.	*	.	-0.60	0.23
Lys	277	.	.	B	B	.	.	.	-1.28	.	*	.	-0.60	0.46
Asn	278	.	.	B	B	.	.	.	-1.07	.	*	.	-0.60	0.39
Ile	279	.	.	B	B	.	.	.	-0.56	.	*	.	0.30	0.91
Thr	280	.	.	B	B	.	.	.	-0.60	.	*	.	0.30	0.45
Ile	281	.	.	B	B	.	.	.	-0.33	.	.	.	-0.30	0.21
Glu	282	.	.	B	B	.	.	.	-0.62	.	*	.	-0.60	0.30
Gly	283	.	.	B	B	.	.	.	-0.93	.	*	.	-0.60	0.32
Val	284	.	.	B	B	.	.	.	-0.34	.	*	.	-0.60	0.67
Ala	285	.	.	B	B	.	.	.	-0.03	.	.	.	-0.30	0.52
Tyr	286	.	.	.	B	T	.	.	0.19	*	.	.	0.10	0.90
Thr	287	T	T	.	-0.51	*	.	F	0.65	0.65
Ser	288	T	T	.	-0.38	.	.	F	0.35	0.56
Glu	289	T	T	.	-0.19	.	.	F	0.35	0.55
Cys	290	.	.	B	.	.	T	.	0.44	.	.	.	0.10	0.20
Phe	291	.	.	B	0.48	.	.	.	0.50	0.31
Pro	292	T	.	.	0.44	.	.	.	0.90	0.27
Cys	293	T	.	.	0.43	.	.	.	0.30	0.50
Lys	294	.	.	B	.	.	T	.	-0.27	.	.	F	0.25	0.84

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Pro	295	T	T	.	0.10	.	.	F	0.65	0.47
Gly	296	T	T	.	0.80	*	*	F	0.80	1.17
Thr	297	T	T	.	1.06	*	.	F	1.25	0.94
Phe	298	.	.	B	1.51	*	.	F	1.08	1.22
Ser	299	.	.	B	1.12	*	.	F	1.36	1.91
Asn	300	T	.	.	1.03	.	.	F	2.04	1.31
Lys	301	T	C	0.68	.	*	F	2.32	2.03
Pro	302	T	T	.	0.99	.	.	F	2.80	1.31
Gly	303	T	T	.	1.02	.	*	F	2.52	1.31
Ser	304	T	T	.	1.32	.	*	F	1.49	0.35
Phe	305	T	.	.	0.47	*	*	.	0.56	0.39
Asn	306	.	.	B	-0.24	.	*	.	-0.12	0.29
Cys	307	.	.	B	-0.24	.	*	.	-0.40	0.12
Gln	308	.	.	B	0.21	.	*	.	-0.40	0.21
Val	309	.	.	B	0.51	.	*	.	-0.10	0.26
Cys	310	.	.	B	.	.	T	.	0.90	.	*	.	0.10	0.77
Pro	311	T	T	.	0.66	.	*	F	1.59	0.64
Arg	312	T	T	.	1.02	.	*	F	1.48	1.35
Asn	313	T	T	.	1.02	.	*	F	1.82	3.38
Thr	314	T	.	.	1.92	.	*	F	2.86	3.79
Tyr	315	T	T	.	2.24	.	.	F	3.40	3.87
Ser	316	T	C	1.87	.	*	F	2.86	2.38
Glu	317	T	T	.	1.80	.	.	F	2.42	1.67
Lys	318	T	T	.	1.80	*	.	F	2.38	2.13
Gly	319	.	A	.	.	T	.	.	1.44	*	.	F	1.64	2.75
Ala	320	.	A	.	.	T	.	.	0.80	*	*	F	1.15	0.85
Lys	321	A	A	1.21	*	*	F	0.75	0.30
Glu	322	.	A	B	0.54	*	*	.	0.60	0.59
Cys	323	.	A	B	0.54	*	*	.	0.94	0.31
Ile	324	.	A	B	0.89	*	*	.	1.28	0.31
Arg	325	.	A	B	1.48	*	*	.	1.62	0.30
Cys	326	.	.	B	.	.	T	.	1.13	*	*	.	2.36	0.94
Lys	327	T	T	.	1.13	.	*	F	3.40	1.80
Asp	328	T	T	.	1.10	.	*	F	3.06	1.59
Asp	329	T	T	.	1.69	.	*	F	2.72	2.57
Ser	330	T	.	.	1.23	.	*	F	2.18	1.72
Gln	331	T	.	.	1.60	.	*	F	1.84	1.02
Phe	332	T	T	.	1.26	.	*	F	1.25	0.82
Ser	333	T	C	1.26	.	*	F	0.45	0.82
Gly	334	T	T	.	0.59	.	*	F	1.55	0.82
Ser	335	T	C	0.58	.	*	F	1.65	0.51
Ser	336	C	0.58	*	*	F	1.75	0.55
Glu	337	T	.	.	1.39	*	*	F	2.55	0.96
Cys	338	T	.	.	1.48	*	*	F	3.00	1.40
Thr	339	T	.	.	1.61	.	*	F	2.70	1.61
Glu	340	T	.	.	1.24	*	*	F	2.74	1.44
Arg	341	C	1.23	*	*	F	2.58	1.44
Pro	342	T	C	0.92	*	*	F	2.82	1.44
Pro	343	T	T	.	1.63	*	*	F	3.06	1.20

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Cys	344	T	T	.	1.94	*	.	F	3.40	1.22
Thr	345	T	T	.	1.70	*	*	F	3.06	1.32
Thr	346	T	T	.	0.89	*	*	F	2.42	1.34
Lys	347	T	T	.	1.10	*	*	F	1.48	2.16
Asp	348	T	T	.	0.42	.	*	F	1.74	2.60
Tyr	349	.	.	B	.	.	T	.	1.06	.	*	.	0.25	1.26
Phe	350	.	.	B	B	.	.	.	1.06	*	*	.	-0.30	0.86
Gln	351	.	.	B	B	.	.	.	1.16	*	*	.	-0.60	0.74
Ile	352	.	.	B	B	.	.	.	0.44	*	*	.	-0.26	0.73
His	353	.	.	B	B	.	.	.	0.44	.	*	.	0.08	0.45
Thr	354	T	C	0.69	.	*	.	1.32	0.44
Pro	355	T	C	1.39	.	*	F	2.56	1.08
Cys	356	T	T	.	1.04	*	*	F	3.40	1.37
Asp	357	T	T	.	1.98	*	*	F	2.91	0.94
Glu	358	T	.	.	1.70	.	*	F	2.52	1.22
Glu	359	T	.	.	2.01	.	*	F	2.18	3.28
Gly	360	T	.	.	1.33	.	*	F	1.84	3.40
Lys	361	A	.	.	B	.	.	.	1.40	.	*	F	0.90	1.38
Thr	362	A	.	.	B	.	.	.	1.16	.	*	F	0.45	0.79
Gln	363	.	.	B	B	.	.	.	1.20	.	*	.	-0.15	1.25
Ile	364	.	.	B	B	.	.	.	0.91	*	*	.	0.45	1.25
Met	365	.	.	B	B	.	.	.	0.37	*	*	.	-0.60	0.91
Tyr	366	.	.	B	B	.	.	.	0.32	*	*	.	-0.60	0.37
Lys	367	.	.	B	B	.	.	.	0.42	*	*	.	-0.60	0.91
Trp	368	.	.	.	B	T	.	.	0.47	*	*	.	-0.05	1.42
Ile	369	.	.	.	B	.	.	C	0.47	.	*	.	0.65	1.81
Glu	370	.	.	.	B	.	.	C	0.40	*	.	.	0.50	0.63
Pro	371	.	A	.	.	T	.	.	0.76	*	.	.	0.10	0.32
Lys	372	.	A	.	.	T	.	.	0.71	*	.	.	1.00	0.90
Ile	373	.	A	B	1.00	.	*	.	0.60	0.90
Cys	374	.	A	B	1.08	*	*	.	0.60	0.98
Arg	375	.	A	B	0.77	*	.	F	0.75	0.40
Glu	376	.	A	B	0.98	*	.	F	0.75	0.83
Asp	377	.	A	B	0.34	*	.	F	0.90	2.58
Leu	378	.	A	B	0.34	*	*	F	0.90	1.33
Thr	379	.	A	.	.	T	.	.	1.12	*	*	F	1.15	0.54
Asp	380	.	A	B	0.20	*	*	F	0.75	0.63
Ala	381	.	A	B	-0.01	*	*	.	-0.30	0.63
Ile	382	.	A	B	-0.22	*	*	.	0.30	0.68
Arg	383	.	.	B	0.29	*	*	.	0.84	0.63
Leu	384	.	.	B	0.26	*	*	.	0.58	0.83
Pro	385	T	C	0.26	*	*	F	1.62	1.17
Pro	386	T	T	.	0.89	*	*	F	3.06	1.04
Ser	387	T	T	.	1.82	*	*	F	3.40	2.52
Gly	388	T	T	.	1.71	*	*	F	3.06	3.26
Glu	389	T	.	.	1.86	.	.	F	2.75	3.52
Lys	390	T	T	.	1.86	.	.	F	2.84	1.41
Lys	391	T	T	.	1.86	.	.	F	2.73	2.20
Asp	392	T	T	.	1.49	.	.	F	2.62	1.96

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Cys	393	.	.	B	.	.	T	.	1.83	.	.	F	2.30	0.53
Pro	394	.	.	B	1.62	.	.	F	1.87	0.42
Pro	395	T	.	.	1.23	*	.	F	1.74	0.39
Cys	396	T	.	.	0.49	.	.	F	0.91	0.72
Asn	397	.	.	B	.	.	T	.	0.24	.	.	F	0.18	0.40
Pro	398	T	T	.	0.91	.	.	F	0.35	0.41
Gly	399	T	T	.	1.12	.	.	.	0.35	1.23
Phe	400	.	.	B	.	.	T	.	0.99	.	.	.	-0.05	1.23
Tyr	401	.	.	B	1.36	.	.	.	-0.40	0.79
Asn	402	T	T	.	1.06	.	.	F	0.63	1.07
Asn	403	T	T	.	0.97	.	.	F	0.76	1.65
Gly	404	T	T	.	0.64	*	.	F	1.19	1.41
Ser	405	T	T	.	1.31	*	.	F	1.77	0.47
Ser	406	T	T	.	1.34	.	.	F	1.30	0.40
Ser	407	T	T	.	0.68	.	.	F	1.17	0.62
Cys	408	.	.	B	.	.	T	.	0.47	.	.	.	0.49	0.25
His	409	.	.	B	.	.	T	.	0.60	.	.	.	0.36	0.29
Pro	410	T	.	.	0.56	.	.	.	0.43	0.33
Cys	411	.	.	B	0.54	.	.	.	-0.10	0.61
Pro	412	.	.	B	.	.	T	.	0.14	.	.	F	0.25	0.65
Pro	413	T	T	.	0.51	.	.	F	0.35	0.36
Gly	414	T	T	.	0.54	.	.	F	0.35	0.91
Thr	415	.	.	B	.	.	T	.	0.41	.	.	F	1.19	0.98
Phe	416	.	.	B	.	.	T	.	0.77	*	.	F	1.53	0.63
Ser	417	.	.	B	.	.	T	.	1.02	*	.	F	1.27	0.92
Asp	418	T	T	.	1.23	*	.	F	2.76	1.27
Gly	419	T	T	.	0.91	*	*	F	3.40	2.54
Thr	420	T	.	.	1.33	*	*	F	2.86	1.02
Lys	421	T	.	.	1.82	*	*	F	2.52	1.19
Glu	422	T	.	.	1.46	*	*	F	2.18	1.86
Cys	423	T	.	.	1.24	*	*	F	1.69	0.69
Arg	424	.	.	B	1.00	*	.	F	0.95	0.53
Pro	425	.	.	B	0.97	*	.	F	0.93	0.31
Cys	426	T	T	.	0.61	*	.	F	1.81	0.58
Pro	427	T	T	.	0.61	*	*	F	2.09	0.42
Ala	428	T	T	.	1.07	*	.	F	2.37	0.47
Gly	429	T	T	.	0.37	*	*	F	2.80	1.37
Thr	430	.	.	B	-0.23	.	.	F	1.77	0.89
Glu	431	.	.	B	0.09	.	.	F	0.89	0.73
Pro	432	.	.	B	-0.40	.	.	F	1.21	0.73
Ala	433	.	.	B	0.19	.	*	.	0.18	0.44
Leu	434	.	.	B	0.29	.	*	.	0.50	0.44
Gly	435	.	.	B	0.64	.	*	.	-0.40	0.44
Phe	436	.	.	B	0.36	.	*	.	-0.10	0.88
Glu	437	.	.	B	0.28	.	*	.	-0.25	1.12
Tyr	438	T	.	.	0.87	.	*	.	0.15	1.19
Lys	439	T	.	.	0.82	*	*	.	0.15	2.21
Trp	440	.	.	.	B	T	.	.	0.36	*	.	.	-0.20	0.95
Trp	441	.	.	B	B	.	.	.	0.84	*	*	.	-0.60	0.50

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Asn	442	.	.	B	B	.	.	.	0.50	.	.	.	-0.60	0.39
Val	443	.	.	.	B	.	.	C	0.74	*	.	.	-0.40	0.36
Leu	444	T	C	0.10	*	*	.	0.00	0.56
Pro	445	T	T	.	0.43	*	*	F	0.52	0.34
Gly	446	T	T	.	0.41	*	*	F	0.99	0.92
Asn	447	T	T	.	0.11	.	*	F	1.31	1.61
Met	448	T	.	.	0.30	.	*	F	1.88	1.40
Lys	449	.	.	B	.	.	T	.	0.41	.	*	F	1.70	0.76
Thr	450	.	.	B	.	.	T	.	0.62	.	*	F	0.93	0.41
Ser	451	.	.	B	.	.	T	.	0.11	.	*	.	0.61	0.66
Cys	452	.	.	B	.	.	T	.	-0.23	*	.	.	0.44	0.25
Phe	453	.	.	B	B	.	.	.	0.37	.	.	.	-0.43	0.17
Asn	454	.	.	B	B	.	.	.	0.02	.	.	.	-0.29	0.20
Val	455	.	.	.	B	T	.	.	0.38	.	.	.	0.42	0.51
Gly	456	T	.	.	0.01	.	.	F	1.53	1.17
Asn	457	T	T	.	0.68	.	*	F	2.49	0.39
Ser	458	T	T	.	1.03	.	*	F	3.10	0.88
Lys	459	T	T	.	0.43	*	.	F	2.79	0.88
Cys	460	T	T	.	1.29	.	.	F	2.48	0.54
Asp	461	T	.	.	1.29	.	*	F	1.97	0.65
Gly	462	T	T	.	1.00	.	*	F	1.86	0.32
Met	463	T	C	1.30	.	*	F	0.45	0.63
Asn	464	T	C	0.40	.	*	.	0.90	0.65
Gly	465	T	C	0.48	*	.	.	0.00	0.49
Trp	466	C	0.13	*	.	.	-0.20	0.50
Glu	467	.	.	B	0.48	*	.	.	-0.10	0.31
Val	468	.	.	B	1.04	*	.	.	0.50	0.52
Ala	469	.	.	B	0.16	*	.	.	0.50	0.67
Gly	470	.	.	B	0.50	*	.	.	0.50	0.27
Asp	471	.	.	B	0.49	*	.	.	-0.10	0.63
His	472	.	.	B	0.14	*	.	F	0.65	0.84
Ile	473	.	.	B	0.41	*	.	F	0.65	0.84
Gln	474	.	.	B	.	.	T	.	0.66	*	.	F	0.85	0.51
Ser	475	.	.	B	.	.	T	.	0.66	*	.	F	0.25	0.37
Gly	476	T	T	.	0.36	*	.	F	0.65	0.52
Ala	477	T	C	0.39	*	.	F	1.35	0.40
Gly	478	C	1.28	.	.	F	1.45	0.50
Gly	479	C	1.28	.	.	F	1.75	0.82
Ser	480	C	1.33	.	.	F	2.50	1.35
Asp	481	T	C	0.87	.	.	F	3.00	2.14
Asn	482	.	.	B	.	.	T	.	0.57	.	.	F	2.20	1.78
Asp	483	.	.	B	.	.	T	.	0.10	.	.	F	1.75	0.93
Tyr	484	.	.	B	.	.	T	.	0.44	.	.	.	0.70	0.46
Leu	485	.	.	B	B	.	.	.	-0.07	.	.	.	-0.30	0.46
Ile	486	.	.	B	B	.	.	.	-0.10	.	*	.	-0.60	0.23
Leu	487	.	.	B	B	.	.	.	-0.99	.	*	.	-0.60	0.20
Asn	488	.	.	B	B	.	.	.	-1.20	.	*	.	-0.60	0.17
Leu	489	.	.	B	B	.	.	.	-1.30	.	*	.	-0.60	0.37
His	490	.	.	B	B	.	.	.	-1.19	*	*	.	-0.60	0.44

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Ile	491	.	.	B	B	.	.	.	-0.26	*	.	.	-0.60	0.24
Pro	492	T	.	.	0.34	*	*	.	0.00	0.58
Gly	493	T	.	.	0.13	.	*	F	0.45	0.66
Phe	494	T	.	.	0.63	.	.	F	0.88	1.45
Lys	495	C	0.37	.	.	F	1.56	1.36
Pro	496	T	C	0.66	.	.	F	2.04	1.84
Pro	497	T	C	0.56	.	*	F	1.72	2.10
Thr	498	T	T	.	0.56	.	*	F	2.80	1.51
Ser	499	.	.	B	.	.	T	.	0.67	.	*	F	1.37	0.97
Met	500	.	.	B	0.31	.	.	F	0.89	0.63
Thr	501	.	.	B	0.18	.	.	F	0.82	0.63
Gly	502	.	.	B	0.09	.	.	F	0.75	0.47
Ala	503	T	C	0.40	.	.	F	1.08	0.63
Thr	504	T	C	-0.11	.	.	F	1.89	0.76
Gly	505	T	C	0.14	*	.	F	2.10	0.63
Ser	506	T	C	0.57	*	*	F	1.89	0.62
Glu	507	.	.	B	0.02	*	*	F	1.58	0.84
Leu	508	.	.	B	B	.	.	.	0.30	*	*	F	0.87	0.60
Gly	509	.	.	B	B	.	.	.	-0.09	*	*	F	0.66	0.64
Arg	510	.	.	B	B	.	.	.	-0.60	*	*	.	-0.30	0.32
Ile	511	.	.	B	B	.	.	.	-1.00	*	*	.	-0.60	0.29
Thr	512	.	.	B	B	.	.	.	-1.00	*	*	.	-0.60	0.25
Phe	513	.	.	B	B	.	.	.	-0.50	*	*	.	-0.30	0.22
Val	514	.	.	B	B	.	.	.	-0.97	*	*	.	-0.60	0.46
Phe	515	.	.	B	B	.	.	.	-1.74	*	*	.	-0.60	0.26
Glu	516	.	.	B	B	.	.	.	-1.16	*	*	.	-0.60	0.16
Thr	517	.	.	.	B	T	.	.	-1.43	.	.	.	-0.20	0.29
Leu	518	.	.	.	B	T	.	.	-0.73	.	.	.	-0.20	0.34
Cys	519	.	.	.	B	T	.	.	-0.54	.	.	.	0.70	0.33
Ser	520	T	T	.	-0.70	*	.	.	0.50	0.12
Ala	521	T	T	.	-1.51	.	.	.	0.50	0.11
Asp	522	T	T	.	-1.44	.	.	.	0.50	0.17
Cys	523	.	.	B	.	.	T	.	-1.33	.	.	.	-0.20	0.20
Val	524	.	.	B	B	.	.	.	-1.27	.	*	.	-0.60	0.17
Leu	525	.	.	B	B	.	.	.	-1.82	*	*	.	-0.60	0.10
Tyr	526	.	.	B	B	.	.	.	-1.23	*	*	.	-0.60	0.14
Phe	527	.	.	B	B	.	.	.	-2.12	*	*	.	-0.60	0.31
Met	528	.	.	B	B	.	.	.	-1.46	*	*	.	-0.60	0.27
Val	529	.	.	B	B	.	.	.	-0.49	*	*	.	-0.26	0.27
Asp	530	.	.	B	B	.	.	.	0.37	*	*	.	0.38	0.62
Ile	531	.	.	B	0.31	*	*	.	1.97	1.25
Asn	532	T	T	.	0.70	*	*	F	3.06	2.27
Arg	533	T	T	.	1.30	*	*	F	3.40	1.96
Lys	534	T	T	.	1.30	*	*	F	3.06	4.49
Ser	535	T	C	0.44	*	.	F	2.52	2.07
Thr	536	.	.	.	B	.	.	C	1.33	*	.	F	1.33	0.79
Asn	537	.	.	B	B	.	.	.	1.03	*	.	F	0.79	0.68
Val	538	.	.	B	B	.	.	.	0.63	*	.	.	0.30	0.68
Val	539	.	.	B	B	.	.	.	0.24	*	.	.	-0.60	0.50

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Glu	540	.	.	B	B	.	.	.	0.20	*	.	.	-0.60	0.30
Ser	541	T	.	.	0.20	.	.	F	0.45	0.41
Trp	542	T	T	.	0.24	.	.	F	1.25	0.79
Gly	543	T	C	1.10	.	.	F	1.95	0.91
Gly	544	T	C	2.00	.	.	F	2.40	1.18
Thr	545	T	C	2.00	.	.	F	3.00	2.24
Lys	546	.	A	C	1.71	.	.	F	2.30	3.93
Glu	547	.	A	C	1.76	.	.	F	2.00	4.01
Lys	548	.	A	.	.	T	.	.	1.79	.	.	F	1.90	4.35
Gln	549	.	A	B	2.10	*	.	F	1.20	3.14
Ala	550	.	A	B	1.52	*	.	.	0.45	2.47
Tyr	551	.	.	B	B	.	.	.	0.59	.	.	.	-0.30	0.87
Thr	552	.	.	B	B	.	.	.	-0.11	.	*	.	-0.60	0.35
His	553	.	.	B	B	.	.	.	-0.11	.	.	.	-0.60	0.30
Ile	554	.	.	B	B	.	.	.	-0.11	*	*	.	-0.60	0.38
Ile	555	.	.	B	B	.	.	.	-0.11	*	.	.	-0.60	0.43
Phe	556	.	.	B	B	.	.	.	-0.18	.	.	.	-0.60	0.32
Lys	557	.	.	B	B	.	.	.	-0.57	.	.	.	-0.60	0.65
Asn	558	.	.	.	B	.	.	C	-0.84	*	*	.	-0.40	0.81
Ala	559	.	.	.	B	.	.	C	-0.66	.	*	.	-0.25	1.34
Thr	560	.	.	.	B	.	.	C	-0.08	*	*	.	-0.40	0.58
Phe	561	.	.	.	B	.	.	C	0.33	*	*	.	-0.40	0.52
Thr	562	.	.	B	B	.	.	.	-0.30	.	*	.	-0.60	0.54
Phe	563	.	A	B	B	.	.	.	-1.00	.	*	.	-0.60	0.38
Thr	564	.	A	B	B	.	.	.	-0.41	*	*	.	-0.60	0.38
Trp	565	.	A	B	B	.	.	.	0.01	*	*	.	-0.60	0.46
Ala	566	.	A	.	B	.	.	C	0.40	.	*	.	-0.25	1.03
Phe	567	.	A	.	B	T	.	.	0.71	*	.	.	-0.05	1.03
Gln	568	.	A	.	B	T	.	.	1.41	*	.	.	0.29	1.58
Arg	569	.	A	.	B	T	.	.	1.38	.	.	F	1.68	2.71
Thr	570	.	.	.	B	T	.	.	1.67	*	.	F	2.02	3.09
Asn	571	T	T	.	2.26	*	.	F	2.76	3.09
Gln	572	T	T	.	2.96	*	.	F	3.40	2.64
Gly	573	T	T	.	3.07	.	.	F	2.76	2.94
Gln	574	T	T	.	3.07	.	*	F	2.98	3.58
Asp	575	C	2.68	*	.	F	2.50	4.05
Asn	576	T	C	1.79	*	.	F	2.62	3.54
Arg	577	.	.	B	.	.	T	.	1.79	*	.	F	2.34	1.43
Arg	578	.	.	B	.	.	T	.	2.13	*	.	F	2.60	1.38
Phe	579	.	.	B	.	.	T	.	1.53	*	.	.	2.19	1.43
Ile	580	.	.	B	B	.	.	.	0.68	*	.	.	1.38	0.72
Asn	581	.	.	B	B	.	.	.	0.72	*	*	.	0.22	0.27
Asp	582	.	.	B	B	.	.	.	-0.28	*	*	.	-0.04	0.63
Met	583	.	.	B	B	.	.	.	-0.63	*	*	.	-0.30	0.63
Val	584	.	.	B	B	.	.	.	-0.23	*	.	.	-0.30	0.62
Lys	585	.	.	B	B	.	.	.	-0.23	*	*	.	-0.30	0.50
Ile	586	.	.	B	B	.	.	.	-0.54	*	.	.	-0.60	0.35
Tyr	587	.	.	B	B	.	.	.	-1.13	.	.	.	-0.60	0.68
Ser	588	.	.	B	B	.	.	.	-0.84	.	.	.	-0.60	0.34

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Ile	589	.	.	B	B	.	.	.	0.01	.	.	.	-0.60	0.71
Thr	590	.	.	B	B	.	.	.	-0.62	.	.	.	-0.60	0.73
Ala	591	.	.	B	B	.	.	.	-0.59	.	.	F	-0.45	0.55
Thr	592	.	.	B	B	.	.	.	-0.34	.	*	F	-0.45	0.58
Asn	593	.	.	B	B	.	.	.	-0.39	*	.	.	-0.30	0.67
Ala	594	.	.	B	.	.	T	.	-0.36	*	.	.	0.70	0.66
Val	595	.	.	B	.	.	T	.	-0.63	*	.	.	0.10	0.34
Asp	596	.	.	B	.	.	T	.	-0.34	*	.	.	0.10	0.21
Gly	597	.	.	B	.	.	T	.	-0.33	*	.	.	0.10	0.28
Val	598	.	.	B	-1.00	*	.	.	0.50	0.51
Ala	599	.	.	B	-0.30	*	*	.	0.50	0.16
Ser	600	.	.	B	.	.	T	.	-0.03	*	*	.	0.70	0.32
Ser	601	.	.	B	.	.	T	.	-0.70	*	*	.	0.70	0.44
Cys	602	.	.	B	.	.	T	.	-0.94	.	*	.	0.70	0.23
Arg	603	.	.	B	.	.	T	.	-0.90	.	*	.	0.70	0.18
Ala	604	.	.	B	-0.66	.	*	.	-0.10	0.11
Cys	605	.	.	B	-0.66	*	*	.	-0.10	0.20
Ala	606	.	.	B	-0.36	*	*	.	-0.10	0.14
Leu	607	.	.	B	0.31	*	*	.	0.24	0.24
Gly	608	.	.	B	.	.	T	.	-0.10	.	*	F	1.53	0.76
Ser	609	.	.	B	.	T	T	.	0.14	.	.	F	2.42	1.01
Glu	610	T	T	.	0.51	.	.	F	2.76	1.21
Gln	611	T	T	.	0.80	.	.	F	3.40	1.64
Ser	612	T	.	.	0.94	.	.	F	2.86	1.64
Gly	613	T	T	.	0.43	.	.	F	2.27	0.51
Ser	614	T	T	.	0.52	.	.	F	1.33	0.22
Ser	615	T	T	.	-0.14	.	.	F	0.99	0.25
Cys	616	.	.	B	.	.	T	.	-0.36	.	.	.	-0.20	0.14
Val	617	.	.	B	-0.27	.	.	.	-0.40	0.16
Pro	618	.	.	B	-0.27	.	.	.	-0.40	0.18
Cys	619	.	.	B	0.00	.	.	.	-0.40	0.33
Pro	620	.	.	B	.	.	T	.	0.06	.	.	F	-0.05	0.61
Pro	621	T	T	.	-0.17	.	.	F	0.35	0.62
Gly	622	T	T	.	0.69	*	.	.	0.20	0.81
His	623	.	.	B	.	.	T	.	0.94	*	.	.	0.10	0.91
Tyr	624	.	A	B	1.61	*	.	.	0.45	1.17
Ile	625	.	A	B	1.51	*	.	.	0.75	2.06
Glu	626	.	A	B	1.72	*	.	.	1.09	2.18
Lys	627	.	A	.	.	T	.	.	2.07	*	.	F	1.98	2.24
Glu	628	.	A	.	.	T	.	.	1.43	*	.	F	2.32	5.53
Thr	629	.	A	.	.	T	.	.	1.72	*	.	F	2.66	1.71
Asn	630	T	T	.	2.61	*	.	F	3.40	1.71
Gln	631	T	T	.	1.94	*	.	F	3.06	1.71
Cys	632	T	T	.	1.69	*	.	F	2.57	0.64
Lys	633	T	T	.	1.48	.	.	F	2.51	0.61
Glu	634	T	.	.	1.79	*	.	F	2.25	0.55
Cys	635	.	.	B	1.48	*	.	F	1.94	1.70
Pro	636	.	.	B	.	.	T	.	1.23	.	.	F	2.42	1.23
Pro	637	T	T	.	1.09	.	.	F	2.80	1.11

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Phe	687	.	.	B	.	.	T	.	0.30	*	.	.	-0.20	0.92
Ser	688	T	T	.	0.81	*	*	.	0.20	0.79
Asn	689	T	C	-0.01	*	.	F	0.45	0.63
Leu	690	.	.	B	.	.	T	.	-0.11	*	.	F	-0.05	0.54
Ser	691	C	-0.41	.	.	F	0.25	0.40
Ser	692	T	C	-0.52	*	.	F	0.45	0.33
Val	693	.	.	B	.	.	T	.	-0.82	*	.	F	-0.05	0.33
Gly	694	.	.	B	.	.	T	.	-0.82	*	.	F	-0.05	0.25
Ser	695	.	.	B	.	.	T	.	-0.36	*	.	.	-0.20	0.30
Leu	696	.	.	B	-0.27	*	.	.	-0.40	0.39
Met	697	.	.	B	-0.27	.	.	.	-0.10	0.62
Asn	698	.	.	B	-0.11	.	.	F	0.05	0.62
Gly	699	T	C	-0.08	.	.	F	0.15	0.65
Pro	700	T	C	-0.08	*	.	F	0.15	0.94
Ser	701	T	C	0.78	*	.	F	0.73	0.79
Phe	702	.	.	B	.	.	T	.	1.03	.	*	F	1.56	1.59
Thr	703	.	.	B	0.72	*	.	F	1.64	1.02
Ser	704	.	.	B	.	.	T	.	1.11	*	.	F	2.12	1.10
Lys	705	T	T	.	1.08	*	.	F	2.80	2.53
Gly	706	T	T	.	0.68	*	*	F	2.52	2.75
Thr	707	T	T	.	1.34	*	*	F	2.24	1.78
Lys	708	.	.	B	B	.	.	.	0.96	*	.	F	1.16	1.21
Tyr	709	.	.	B	B	.	.	.	0.56	*	.	.	-0.17	1.06
Phe	710	.	.	B	B	.	.	.	0.51	.	*	.	-0.60	0.63
His	711	.	.	B	B	.	.	.	-0.03	.	.	.	-0.60	0.51
Phe	712	.	.	B	B	.	.	.	-0.02	*	*	.	-0.60	0.23
Phe	713	.	.	B	B	.	.	.	-0.88	*	*	.	-0.60	0.35
Asn	714	.	.	.	B	T	.	.	-1.30	.	*	.	-0.20	0.21
Ile	715	.	.	.	B	T	.	.	-0.94	.	*	.	-0.20	0.13
Ser	716	.	.	.	B	.	.	C	-0.94	.	*	.	-0.40	0.15
Leu	717	.	.	.	B	.	.	C	-0.24	.	*	.	-0.06	0.13
Cys	718	T	T	.	0.11	.	*	.	1.18	0.32
Gly	719	T	T	.	0.16	*	*	.	1.52	0.23
His	720	T	T	.	1.09	*	.	.	2.46	0.57
Glu	721	T	T	.	0.79	*	.	F	3.40	2.12
Gly	722	.	A	.	.	T	.	.	1.01	.	.	F	2.66	2.12
Lys	723	.	A	.	.	T	.	.	0.87	*	.	F	2.32	1.57
Lys	724	.	A	B	0.54	*	.	F	1.43	0.75
Met	725	.	A	B	0.27	.	.	.	0.64	0.41
Ala	726	.	A	B	0.27	*	.	.	0.30	0.29
Leu	727	.	A	B	0.61	*	.	.	-0.30	0.24
Cys	728	.	.	B	.	.	T	.	-0.32	*	.	.	-0.20	0.38
Thr	729	.	.	B	.	.	T	.	-0.68	*	.	.	-0.20	0.27
Asn	730	.	.	B	.	.	T	.	-0.08	*	.	F	-0.05	0.46
Asn	731	T	T	.	-0.19	*	.	F	0.80	1.45
Ile	732	.	.	B	B	.	.	.	0.31	.	.	F	-0.15	0.87
Thr	733	.	.	B	B	.	.	.	0.12	*	*	F	-0.15	0.78
Asp	734	.	.	B	B	.	.	.	0.48	*	*	F	-0.45	0.36
Phe	735	.	.	B	B	.	.	.	0.48	*	.	.	-0.15	1.02

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Thr	736	.	.	B	B	.	.	.	-0.41	*	.	.	0.75	1.23
Val	737	.	.	B	B	.	.	.	-0.38	.	.	.	0.30	0.52
Lys	738	.	.	B	B	.	.	.	-0.66	.	*	.	-0.30	0.44
Glu	739	.	.	B	B	.	.	.	-1.00	.	*	.	0.30	0.31
Ile	740	.	.	B	B	.	.	.	-0.60	.	*	.	0.30	0.41
Val	741	.	.	B	B	.	.	.	-0.29	*	.	.	0.30	0.28
Ala	742	.	.	B	B	.	.	.	0.57	*	.	.	0.64	0.27
Gly	743	.	.	B	B	.	.	.	0.28	*	.	F	1.13	0.64
Ser	744	T	C	-0.03	*	.	F	2.22	1.34
Asp	745	T	T	.	0.86	*	.	F	2.76	1.92
Asp	746	T	T	.	0.90	*	.	F	3.40	3.12
Tyr	747	.	.	B	.	.	T	.	0.63	*	.	F	2.36	1.92
Thr	748	.	.	B	B	.	.	.	0.63	*	.	F	1.47	0.85
Asn	749	.	.	B	B	.	.	.	0.34	*	.	.	0.08	0.50
Leu	750	.	.	B	B	.	.	.	-0.36	*	.	.	-0.26	0.33
Val	751	.	.	B	B	.	.	.	-1.21	*	.	.	-0.60	0.20
Gly	752	.	.	B	B	.	.	.	-1.63	*	.	.	-0.60	0.09
Ala	753	.	.	B	B	.	.	.	-1.32	.	.	.	-0.60	0.06
Phe	754	.	.	B	B	.	.	.	-1.62	.	.	.	-0.60	0.14
Val	755	.	.	B	B	.	.	.	-1.12	.	.	.	-0.60	0.19
Cys	756	.	.	B	.	.	T	.	-1.16	.	.	.	-0.20	0.26
Gln	757	.	.	B	.	.	T	.	-1.70	.	.	.	-0.20	0.21
Ser	758	.	.	B	.	.	T	.	-1.32	.	.	F	-0.05	0.20
Thr	759	.	.	B	.	.	T	.	-0.92	.	.	F	-0.05	0.58
Ile	760	.	.	B	-0.07	.	.	F	-0.25	0.45
Ile	761	.	.	B	.	.	T	.	0.30	.	.	F	0.59	0.58
Pro	762	.	.	B	.	.	T	.	0.34	.	*	F	0.93	0.54
Ser	763	T	T	.	0.30	*	.	F	2.42	1.55
Glu	764	T	C	-0.09	*	.	F	2.86	2.18
Ser	765	T	T	.	0.91	*	.	F	3.40	1.22
Lys	766	T	T	.	1.21	*	*	F	3.06	1.79
Gly	767	T	T	.	0.83	*	*	F	2.72	1.04
Phe	768	.	.	B	.	.	T	.	0.32	*	*	.	1.38	0.79
Arg	769	.	.	B	0.02	*	*	.	0.84	0.32
Ala	770	.	.	B	0.02	*	*	.	-0.10	0.44
Ala	771	.	.	B	-0.02	*	*	.	-0.10	0.68
Leu	772	T	C	0.02	*	*	.	0.90	0.60
Ser	773	T	C	-0.17	*	*	F	0.45	0.80
Ser	774	T	C	-1.17	*	*	F	0.15	0.55
Gln	775	.	.	B	.	.	T	.	-1.39	.	.	F	-0.05	0.47
Ser	776	.	.	B	B	.	.	.	-1.39	.	.	F	-0.45	0.29
Ile	777	.	.	B	B	.	.	.	-0.58	.	.	.	-0.60	0.22
Ile	778	.	.	B	B	.	.	.	-0.59	.	.	.	-0.30	0.21
Leu	779	.	.	B	B	.	.	.	-0.99	.	.	.	-0.60	0.23
Ala	780	.	.	B	B	.	.	.	-1.88	.	.	.	-0.60	0.28
Asp	781	.	.	B	B	.	.	.	-1.92	.	.	.	-0.60	0.28
Thr	782	.	.	B	B	.	.	.	-1.89	.	.	.	-0.60	0.34
Phe	783	.	.	B	B	.	.	.	-1.31	.	*	.	-0.60	0.25
Ile	784	.	.	B	B	.	.	.	-1.36	*	*	.	-0.60	0.21

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Gly	785	.	.	B	B	.	.	.	-0.77	.	*	.	-0.60	0.11
Val	786	.	.	B	B	.	.	.	-1.08	.	*	.	-0.60	0.22
Thr	787	.	.	B	B	.	.	.	-1.08	.	*	.	-0.30	0.45
Val	788	.	.	B	B	.	.	.	-1.19	*	*	.	-0.30	0.66
Glu	789	.	.	B	B	.	.	.	-0.26	*	*	F	-0.45	0.73
Thr	790	.	.	B	B	.	.	.	0.09	*	*	F	0.60	1.01
Thr	791	.	.	B	B	.	.	.	0.06	*	*	F	0.60	2.19
Leu	792	.	.	B	B	.	.	.	0.37	*	*	F	0.45	0.89
Lys	793	.	.	.	B	.	.	C	0.33	*	*	F	0.05	0.99
Asn	794	.	.	.	B	.	.	C	0.38	.	*	F	0.05	0.48
Ile	795	.	.	.	B	.	.	C	0.69	.	*	F	0.80	1.17
Asn	796	.	A	B	B	.	.	.	1.00	.	*	F	0.90	1.01
Ile	797	.	A	B	B	.	.	.	1.21	.	*	F	0.90	1.05
Lys	798	.	A	B	B	.	.	.	0.47	.	*	F	0.90	1.48
Glu	799	.	A	B	0.26	.	*	F	0.75	0.80
Asp	800	.	A	B	0.29	.	*	F	0.60	1.76
Met	801	.	A	B	B	.	.	.	0.08	*	*	.	0.60	0.65
Phe	802	.	A	B	B	.	.	.	0.66	.	*	.	0.30	0.58
Pro	803	.	A	B	B	.	.	.	0.31	.	.	.	-0.30	0.50
Val	804	.	.	.	B	.	.	C	0.31	*	.	.	-0.40	0.68
Pro	805	T	C	-0.58	*	.	F	0.30	1.36
Thr	806	T	T	.	-0.19	*	.	F	0.35	0.62
Ser	807	T	C	0.51	*	.	F	0.30	1.29
Gln	808	.	.	B	.	.	T	.	-0.13	.	.	F	1.00	1.39
Ile	809	.	.	B	B	.	.	.	0.69	.	.	F	-0.15	0.71
Pro	810	.	.	B	B	.	.	.	0.20	.	.	F	-0.15	0.73
Asp	811	.	.	B	B	.	.	.	-0.19	.	.	F	-0.45	0.36
Val	812	.	.	B	B	.	.	.	-0.13	.	*	.	-0.56	0.45
His	813	.	.	B	B	.	.	.	-0.09	.	*	.	-0.52	0.45
Phe	814	.	.	B	B	.	.	.	0.50	*	*	.	-0.48	0.54
Phe	815	.	.	B	B	.	.	.	0.41	.	*	.	-0.44	0.98
Tyr	816	T	T	.	0.10	.	*	.	0.40	0.97
Lys	817	T	T	.	0.37	*	*	F	0.66	1.61
Ser	818	T	T	.	0.09	.	.	F	0.92	1.88
Ser	819	T	T	.	0.48	.	.	F	0.88	1.73
Thr	820	.	.	.	B	T	.	.	0.88	.	.	F	1.04	1.25
Ala	821	.	.	.	B	T	.	.	0.46	.	.	F	0.40	1.25
Thr	822	.	.	B	B	.	.	.	-0.48	*	.	F	-0.15	0.50
Thr	823	.	.	B	B	.	.	.	-0.18	.	.	F	-0.45	0.24
Ser	824	.	.	B	B	.	.	.	-0.22	.	*	.	-0.35	0.39
Cys	825	.	.	B	.	.	T	.	0.20	.	*	.	0.30	0.27
Ile	826	.	.	B	.	.	T	.	0.49	.	*	.	1.45	0.36
Asn	827	T	T	.	0.49	.	*	F	2.25	0.36
Gly	828	T	T	.	0.21	.	*	F	2.50	0.97
Arg	829	.	.	.	B	T	.	.	-0.34	.	*	F	2.00	1.40
Ser	830	.	.	.	B	.	.	C	0.37	.	*	F	1.40	0.64
Thr	831	.	A	B	B	.	.	.	0.66	.	*	F	1.40	1.30
Ala	832	.	A	B	B	.	.	.	0.77	.	*	F	0.70	0.66
Val	833	.	A	B	B	.	.	.	0.44	.	*	.	0.30	0.96

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Lys	834	.	A	B	B	.	.	.	0.33	.	*	.	0.30	0.36
Met	835	.	A	B	0.42	.	*	.	0.64	0.57
Arg	836	.	A	B	0.42	.	*	.	1.13	1.18
Cys	837	.	.	B	1.06	.	*	.	1.52	0.85
Asn	838	.	.	B	.	.	T	.	1.61	.	*	F	2.36	1.73
Pro	839	T	T	.	1.22	.	*	F	3.40	1.18
Thr	840	T	T	.	1.23	.	*	F	2.76	2.18
Lys	841	T	T	.	0.78	.	*	F	2.42	1.37
Ser	842	C	0.59	*	.	F	1.53	0.88
Gly	843	.	.	B	.	.	T	.	-0.30	*	.	F	1.19	0.45
Ala	844	.	.	B	.	.	T	.	-0.39	*	.	F	0.25	0.16
Gly	845	.	.	B	.	.	T	.	-0.93	*	.	.	-0.20	0.16
Val	846	.	.	B	.	.	T	.	-1.19	*	.	.	-0.20	0.12
Ile	847	.	.	B	-1.19	*	*	.	-0.40	0.18
Ser	848	.	.	B	-0.80	*	*	.	-0.15	0.25
Val	849	.	.	B	.	.	T	.	-0.88	*	*	F	0.75	0.66
Pro	850	.	.	B	.	.	T	.	-0.74	.	*	F	1.00	0.51
Ser	851	T	T	.	-0.48	*	*	F	2.25	0.58
Lys	852	T	T	.	0.07	.	*	F	2.50	0.80
Cys	853	.	.	B	.	.	T	.	0.06	.	*	F	1.85	0.51
Pro	854	T	T	.	0.24	.	*	F	2.00	0.55
Ala	855	T	T	.	0.46	.	.	F	1.75	0.15
Gly	856	T	T	.	0.41	.	.	F	1.63	0.46
Thr	857	.	.	B	-0.30	.	.	F	0.91	0.29
Cys	858	.	.	B	.	.	T	.	0.06	.	.	F	1.24	0.16
Asp	859	T	T	.	-0.43	.	.	F	1.77	0.23
Gly	860	T	T	.	-0.09	.	.	F	1.30	0.14
Cys	861	.	.	B	.	.	T	.	-0.44	.	.	.	0.32	0.40
Thr	862	.	.	B	-0.94	.	.	.	-0.01	0.21
Phe	863	.	A	B	-0.57	.	.	.	-0.34	0.17
Tyr	864	.	A	B	-0.57	.	.	.	-0.47	0.34
Phe	865	.	A	B	-0.52	.	.	.	-0.60	0.40
Leu	866	.	A	B	-0.44	*	.	.	-0.60	0.63
Trp	867	.	A	C	-0.13	.	.	.	-0.40	0.40
Glu	868	.	A	C	-0.02	*	.	.	-0.10	0.81
Ser	869	.	A	.	.	T	.	.	-0.44	.	.	.	0.70	0.99
Ala	870	.	A	.	.	T	.	.	0.04	.	.	.	0.80	0.50
Glu	871	.	A	.	.	T	.	.	0.04	.	.	.	1.20	0.45
Ala	872	.	A	.	.	T	.	.	-0.33	.	.	.	0.40	0.28
Cys	873	T	C	-0.64	.	.	.	0.70	0.15
Pro	874	T	T	.	-0.34	.	.	.	1.00	0.12
Leu	875	T	T	.	0.21	.	.	.	0.90	0.21
Cys	876	A	T	.	0.21	.	*	.	0.40	0.53
Thr	877	A	A	0.10	.	*	.	0.50	0.58
Glu	878	A	A	0.73	.	*	.	-0.20	0.60
His	879	A	A	0.94	.	.	.	0.45	1.53
Asp	880	.	A	.	.	T	.	.	0.87	.	.	.	1.15	1.84
Phe	881	A	A	1.53	.	.	.	0.60	0.75
His	882	A	A	1.50	.	.	.	0.60	0.95

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Glu	883	A	A	0.91	*	.	.	0.60	0.56
Ile	884	A	A	0.28	*	*	.	0.58	0.66
Glu	885	A	A	0.32	*	.	.	0.86	0.26
Gly	886	.	A	.	.	T	.	.	1.13	*	.	.	1.84	0.30
Ala	887	.	A	.	.	T	.	.	0.82	*	.	.	2.12	0.83
Cys	888	T	T	.	0.12	*	*	.	2.80	0.48
Lys	889	T	T	.	1.01	*	.	F	2.37	0.42
Arg	890	T	T	.	1.01	*	.	F	2.09	0.71
Gly	891	T	T	.	1.04	*	.	F	2.26	2.31
Phe	892	.	.	B	B	.	.	.	0.82	*	.	F	1.18	1.67
Gln	893	.	.	B	B	.	.	.	1.24	*	.	F	0.45	0.70
Glu	894	.	.	B	B	.	.	.	0.34	*	.	F	-0.30	1.11
Thr	895	.	.	B	B	.	.	.	-0.06	*	.	.	-0.60	0.95
Leu	896	.	.	B	B	.	.	.	0.29	*	.	.	-0.60	0.58
Tyr	897	.	.	B	B	.	.	.	0.99	*	.	.	-0.60	0.54
Val	898	.	.	.	B	T	.	.	0.78	.	.	.	-0.20	0.64
Trp	899	.	.	.	B	T	.	.	0.82	.	*	.	-0.05	1.21
Asn	900	.	.	.	B	.	.	C	0.84	.	*	.	0.05	1.54
Glu	901	.	.	.	B	.	.	C	0.99	.	*	F	0.20	2.18
Pro	902	T	T	.	0.34	*	*	F	0.80	1.11
Lys	903	T	T	.	1.24	*	*	F	0.65	0.49
Trp	904	T	T	.	1.19	*	*	.	1.10	0.56
Cys	905	.	.	B	.	.	T	.	0.30	*	*	.	0.10	0.36
Ile	906	.	.	B	B	.	.	.	0.00	*	*	.	-0.30	0.13
Lys	907	.	.	B	B	.	.	.	-0.60	*	*	.	-0.60	0.16
Gly	908	.	.	B	B	.	.	.	-0.86	*	*	.	-0.60	0.25
Ile	909	.	.	B	B	.	.	.	-0.57	*	*	.	-0.30	0.54
Ser	910	.	A	C	0.14	*	.	F	0.65	0.47
Leu	911	.	A	C	1.08	*	.	F	0.65	0.95
Pro	912	.	A	C	0.22	*	.	F	1.10	2.71
Glu	913	.	A	.	.	T	.	.	-0.02	*	.	F	1.30	1.67
Lys	914	.	A	.	.	T	.	.	0.56	*	.	F	1.30	2.05
Lys	915	.	A	.	.	T	.	.	0.19	.	.	F	1.30	1.91
Leu	916	.	A	C	1.00	.	.	F	0.95	0.59
Ala	917	.	A	B	0.90	.	.	.	0.60	0.51
Thr	918	.	.	B	B	.	.	.	0.04	.	.	.	0.30	0.37
Cys	919	.	.	B	B	.	.	.	0.00	.	*	.	-0.30	0.33
Glu	920	.	.	B	B	.	.	.	-0.74	.	.	.	0.30	0.55
Thr	921	.	.	B	B	.	.	.	-0.22	.	*	.	0.30	0.33
Val	922	.	.	B	B	.	.	.	-0.44	*	*	.	-0.60	0.65
Asp	923	.	.	B	B	.	.	.	-0.09	*	*	.	-0.60	0.31
Phe	924	.	.	B	B	.	.	.	-0.28	*	*	.	-0.60	0.43
Trp	925	.	.	B	B	.	.	.	-0.62	*	*	.	-0.60	0.43
Leu	926	.	.	B	B	.	.	.	-0.90	*	*	.	-0.60	0.25
Lys	927	.	.	B	B	.	.	.	-0.39	*	*	.	-0.60	0.29
Val	928	.	.	B	B	.	.	.	-1.24	*	*	.	-0.60	0.28
Gly	929	T	C	-0.89	.	*	.	0.30	0.25
Ala	930	T	C	-1.19	*	*	.	0.30	0.12

Table I (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Gly	931	T	C	-1.08	.	*	.	0.00	0.17
Val	932	.	.	B	.	.	T	.	-1.43	.	*	.	-0.20	0.15
Gly	933	.	.	B	B	.	.	.	-1.17	.	.	.	-0.60	0.21
Ala	934	.	.	B	B	.	.	.	-1.68	.	.	.	-0.60	0.21
Phe	935	.	.	B	B	.	.	.	-1.90	.	.	.	-0.60	0.21
Thr	936	.	.	B	B	.	.	.	-2.37	.	.	.	-0.60	0.18
Ala	937	.	.	B	B	.	.	.	-2.37	.	.	.	-0.60	0.15
Val	938	.	.	B	B	.	.	.	-2.61	.	.	.	-0.60	0.13
Leu	939	.	.	B	B	.	.	.	-2.83	.	.	.	-0.60	0.09
Leu	940	.	.	B	B	.	.	.	-2.44	.	.	.	-0.60	0.07
Val	941	.	.	B	B	.	.	.	-2.80	.	.	.	-0.60	0.14
Ala	942	.	.	B	B	.	.	.	-2.46	.	.	.	-0.60	0.09
Leu	943	.	.	B	B	.	.	.	-2.30	.	.	.	-0.60	0.17
Thr	944	.	.	B	B	.	.	.	-1.78	*	.	.	-0.60	0.20
Cys	945	.	.	B	B	.	.	.	-0.92	*	.	.	-0.60	0.21
Tyr	946	.	.	B	B	.	.	.	-0.02	.	.	.	-0.60	0.51
Phe	947	.	.	.	B	T	.	.	0.57	.	.	.	0.14	0.70
Trp	948	A	.	.	B	.	.	.	1.38	*	.	.	0.23	2.10
Lys	949	A	T	.	1.73	*	.	F	1.42	2.32
Lys	950	A	.	.	.	T	T	.	2.44	.	.	F	2.76	5.37
Asn	951	T	T	.	2.73	*	.	F	3.40	10.21
Gln	952	T	T	.	3.48	*	.	F	3.06	10.21
Lys	953	.	A	.	.	T	.	.	3.46	*	.	F	2.32	10.21
Lys	954	.	A	.	.	T	.	.	2.52	*	.	F	1.98	9.16
Lys	955	.	A	.	.	T	.	.	1.67	*	.	F	1.64	3.71
Lys	956	.	A	B	1.67	*	.	F	0.90	1.53
Thr	957	.	A	B	0.86	*	.	F	0.90	1.23
Ile	958	.	A	B	0.11	*	.	.	0.30	0.51
Leu	959	.	A	B	0.07	*	.	.	-0.60	0.22
Asn	960	.	A	B	-0.37	*	.	.	-0.60	0.24
Leu	961	.	A	B	-0.80	*	.	.	-0.60	0.45
Phe	962	.	A	B	-0.88	*	.	.	-0.60	0.69
Asn	963	.	A	.	.	T	.	.	-0.38	*	.	.	-0.20	0.55

Table II

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Met	1	.	A	B	-0.64	.	*	.	-0.30	0.52
Leu	2	.	A	B	-0.14	.	*	.	-0.60	0.41
Phe	3	.	A	B	-0.10	.	*	.	-0.30	0.63
Arg	4	.	A	B	0.08	.	*	.	-0.30	0.63
Ala	5	T	T	.	-0.39	*	*	F	1.40	1.18
Arg	6	.	.	B	.	.	T	.	0.32	*	*	F	1.00	1.01
Gly	7	T	C	0.79	.	*	F	1.50	1.01
Pro	8	T	C	1.60	.	*	F	1.35	0.99
Val	9	T	.	.	1.14	.	*	F	1.66	0.99
Arg	10	.	.	B	1.44	*	*	F	1.57	0.99
Gly	11	T	T	.	0.99	*	*	F	2.18	0.67
Arg	12	T	T	.	1.44	*	*	F	2.49	0.90
Gly	13	T	T	.	1.44	*	*	F	3.10	0.90
Trp	14	T	T	.	1.71	*	*	F	2.64	1.40
Gly	15	C	1.60	*	*	F	1.78	0.72
Arg	16	.	A	C	1.36	*	*	F	1.42	1.26
Pro	17	.	A	C	1.03	*	*	F	1.45	1.21
Ala	18	.	A	C	1.49	*	.	F	1.78	1.90
Glu	19	.	A	B	1.89	*	.	F	1.92	1.90
Ala	20	.	A	B	1.89	*	*	F	2.26	2.40
Pro	21	T	T	.	1.89	*	*	F	3.40	2.35
Arg	22	T	T	.	1.80	*	*	F	3.06	2.66
Arg	23	T	T	.	2.18	*	*	F	2.72	3.53
Gly	24	T	T	.	1.97	*	*	F	2.66	3.53
Arg	25	T	.	.	2.27	*	*	F	2.40	2.79
Ser	26	T	C	2.18	*	.	F	2.04	1.50
Pro	27	T	C	1.86	*	.	F	2.32	2.03
Pro	28	T	T	.	1.16	.	*	F	2.80	1.60
Trp	29	T	T	.	1.21	.	.	F	1.62	1.21
Ser	30	T	C	0.21	.	.	F	0.99	0.82
Pro	31	T	T	.	-0.16	.	.	.	0.76	0.37
Ala	32	T	T	.	-0.61	.	.	.	0.48	0.19
Trp	33	.	.	B	.	.	T	.	-0.69	.	.	.	-0.20	0.08
Ile	34	.	.	B	-0.99	.	.	.	-0.40	0.05
Cys	35	.	A	B	-1.50	.	.	.	-0.60	0.05
Cys	36	.	A	B	-1.88	.	.	.	-0.60	0.04
Trp	37	.	A	B	-1.63	.	.	.	-0.60	0.06
Ala	38	A	A	-2.01	.	.	.	-0.60	0.11
Leu	39	.	A	.	.	T	.	.	-1.12	.	.	.	-0.20	0.11
Ala	40	.	A	.	.	T	.	.	-1.04	.	.	.	-0.20	0.18
Gly	41	.	A	.	.	T	.	.	-0.97	.	.	.	-0.20	0.18
Cys	42	A	A	-0.97	.	.	.	-0.60	0.22
Gln	43	A	A	-0.97	.	.	.	-0.60	0.23
Ala	44	A	A	-0.50	.	.	.	-0.60	0.23
Ala	45	.	A	B	0.09	.	*	.	-0.60	0.43
Trp	46	.	.	B	.	.	T	.	-0.38	.	*	.	0.10	0.41
Ala	47	.	.	B	.	.	T	.	0.08	.	*	.	-0.20	0.34
Gly	48	.	.	B	.	.	T	.	-0.22	.	*	.	-0.20	0.51
Asp	49	T	C	0.07	.	.	F	0.45	0.65

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Leu	50	T	C	0.36	.	.	F	1.39	0.87
Pro	51	T	C	0.34	*	*	F	1.88	1.17
Ser	52	T	T	.	1.04	*	.	F	2.27	0.94
Ser	53	T	T	.	1.18	*	.	F	2.76	2.24
Ser	54	T	T	.	0.37	*	.	F	3.40	2.24
Ser	55	T	T	.	0.97	*	.	F	2.76	1.38
Arg	56	T	C	0.97	*	.	F	2.22	1.59
Pro	57	T	T	.	0.60	*	.	F	2.36	1.83
Leu	58	C	0.90	*	.	F	1.75	0.73
Pro	59	T	C	1.20	*	*	F	1.89	0.65
Pro	60	T	T	.	1.54	*	.	F	2.37	0.73
Cys	61	T	T	.	1.43	*	.	F	2.80	1.76
Gln	62	A	T	.	1.40	.	.	F	2.42	1.90
Glu	63	A	2.18	.	.	F	1.94	1.93
Lys	64	A	1.69	.	.	F	1.66	4.90
Asp	65	A	1.90	.	*	F	1.38	2.45
Tyr	66	T	.	.	2.32	.	*	.	1.35	2.45
His	67	A	2.01	.	.	.	0.65	1.92
Phe	68	A	2.01	.	.	.	0.05	1.66
Glu	69	A	1.30	.	.	.	0.05	1.83
Tyr	70	A	1.30	.	*	.	0.21	0.72
Thr	71	A	1.24	.	.	.	1.27	1.39
Glu	72	T	.	.	0.98	.	.	F	2.43	1.08
Cys	73	T	.	.	1.33	.	.	F	2.29	0.92
Asp	74	T	T	.	1.03	*	.	F	3.10	0.63
Ser	75	T	T	.	1.39	*	.	F	2.79	0.49
Ser	76	T	T	.	1.41	*	*	F	2.63	1.79
Gly	77	T	T	.	1.52	.	*	F	2.02	1.12
Ser	78	.	.	.	B	T	.	.	1.33	.	*	F	1.31	1.64
Arg	79	.	.	.	B	T	.	.	0.74	*	*	F	0.85	0.91
Trp	80	.	.	B	B	.	.	.	0.16	.	*	.	0.30	0.93
Arg	81	.	.	B	B	.	.	.	0.24	*	*	.	-0.30	0.49
Val	82	.	.	B	B	.	.	.	0.59	*	*	.	-0.30	0.38
Ala	83	.	.	B	B	.	.	.	0.59	*	*	.	-0.60	0.59
Ile	84	.	.	B	.	.	T	.	-0.11	.	*	.	0.10	0.40
Pro	85	T	C	-0.68	.	*	F	0.15	0.55
Asn	86	T	T	.	-0.79	.	*	F	0.35	0.40
Ser	87	.	.	B	.	T	T	.	-0.60	.	.	F	0.65	0.96
Ala	88	.	.	B	-0.31	.	.	.	0.50	0.33
Val	89	.	.	B	0.23	.	.	.	0.50	0.28
Asp	90	.	.	B	-0.37	.	.	.	0.50	0.20
Cys	91	.	.	B	.	.	T	.	-0.58	.	.	.	0.10	0.17
Ser	92	.	.	B	.	.	T	.	-0.28	.	.	F	0.25	0.35
Gly	93	.	.	B	.	.	T	.	0.10	.	.	F	0.85	0.35
Leu	94	T	C	0.10	*	*	F	1.54	1.00
Pro	95	C	0.21	*	*	F	0.93	0.55
Asp	96	T	C	0.53	*	*	F	2.22	1.10
Pro	97	.	.	B	.	.	T	.	0.88	*	*	F	2.36	1.32
Val	98	T	T	.	1.22	*	*	F	3.40	1.70

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Arg	99	.	.	B	.	.	T	.	1.37	*	*	F	2.66	1.77
Gly	100	T	T	.	1.27	*	*	F	2.57	0.61
Lys	101	T	T	.	0.57	*	*	F	2.38	1.19
Glu	102	.	.	B	.	.	T	.	0.48	.	*	F	1.49	0.53
Cys	103	.	.	B	.	.	T	.	0.67	*	*	.	0.70	0.71
Thr	104	.	.	B	-0.03	*	*	.	0.50	0.19
Phe	105	.	.	B	0.01	.	.	.	-0.10	0.11
Ser	106	.	.	B	-0.38	.	.	.	-0.40	0.28
Cys	107	T	T	.	-0.38	.	.	.	0.20	0.19
Ala	108	A	T	.	0.04	.	.	.	0.10	0.38
Ser	109	A	T	.	-0.46	.	.	F	0.25	0.45
Gly	110	A	T	.	0.24	.	.	F	0.25	0.69
Glu	111	A	A	-0.06	.	*	F	0.60	1.18
Tyr	112	A	A	0.66	.	.	.	0.30	0.87
Leu	113	A	A	1.24	.	*	.	0.75	1.76
Glu	114	A	A	1.54	.	*	.	0.75	1.63
Met	115	A	A	1.03	.	*	.	0.45	1.80
Lys	116	A	A	0.37	.	*	F	0.60	1.62
Asn	117	A	A	0.31	.	*	F	0.45	0.50
Gln	118	A	A	1.17	.	*	F	-0.15	0.68
Val	119	A	A	0.50	.	*	.	0.60	0.68
Cys	120	.	A	B	0.76	.	*	.	0.61	0.23
Ser	121	.	.	B	.	.	T	.	0.71	.	*	.	0.72	0.13
Lys	122	.	.	B	.	.	T	.	0.37	*	.	F	1.78	0.30
Cys	123	.	.	B	.	.	T	.	0.06	*	.	F	2.39	0.56
Gly	124	T	T	.	0.67	*	.	F	3.10	0.60
Glu	125	T	.	.	1.03	*	.	F	2.29	0.47
Gly	126	.	.	B	.	.	T	.	0.52	*	.	F	1.33	1.18
Thr	127	.	.	B	.	.	T	.	0.13	*	.	F	0.87	0.98
Tyr	128	.	.	B	.	.	T	.	0.50	.	.	F	0.56	0.56
Ser	129	.	.	B	.	.	T	.	0.50	*	*	.	-0.20	0.76
Leu	130	.	.	B	-0.39	*	*	F	-0.25	0.52
Gly	131	T	T	.	0.00	*	*	F	0.35	0.23
Ser	132	T	C	-0.39	.	*	F	0.45	0.35
Gly	133	T	C	-0.14	.	*	F	0.15	0.37
Ile	134	T	C	0.16	.	*	F	1.05	0.62
Lys	135	.	A	B	0.68	.	*	F	0.75	0.80
Phe	136	.	A	B	1.02	.	.	F	0.45	0.85
Asp	137	.	A	B	1.32	.	*	F	0.90	2.02
Glu	138	A	A	0.86	.	*	F	0.90	1.75
Trp	139	A	A	1.53	.	*	F	0.90	1.66
Asp	140	A	A	0.90	.	*	F	0.90	1.54
Glu	141	A	A	1.26	*	.	F	0.45	0.90
Leu	142	A	T	.	0.56	*	.	.	0.70	0.85
Pro	143	A	T	.	0.26	*	.	.	0.70	0.44
Ala	144	T	T	.	0.54	*	.	.	0.50	0.34
Gly	145	A	T	.	-0.34	*	.	.	-0.20	0.66
Phe	146	A	-0.93	*	.	.	-0.40	0.30
Ser	147	A	-0.43	*	.	.	-0.40	0.30

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Asn	148	A	.	.	B	.	.	.	-0.92	*	.	.	-0.60	0.44
Ile	149	.	.	B	B	.	.	.	-0.93	*	.	.	-0.60	0.44
Ala	150	.	.	B	B	.	.	.	-0.59	*	.	.	-0.60	0.32
Thr	151	.	.	B	B	.	.	.	-0.20	*	.	.	-0.60	0.34
Phe	152	.	.	B	B	.	.	.	-0.76	*	.	.	-0.60	0.69
Met	153	.	.	B	B	.	.	.	-1.61	*	.	.	-0.60	0.51
Asp	154	.	.	B	B	.	.	.	-1.07	*	.	.	-0.60	0.26
Thr	155	.	.	B	B	.	.	.	-0.69	.	.	.	-0.60	0.30
Val	156	.	.	B	B	.	.	.	-0.68	*	.	.	0.04	0.47
Val	157	.	.	B	B	.	.	.	0.02	*	.	F	0.53	0.37
Gly	158	T	C	0.32	.	*	F	1.47	0.43
Pro	159	T	T	.	0.43	.	*	F	2.61	0.78
Ser	160	T	T	.	0.53	.	*	F	3.40	2.06
Asp	161	T	T	.	1.39	.	*	F	3.06	3.22
Ser	162	.	.	B	1.90	.	*	F	2.43	3.48
Arg	163	.	.	B	.	.	T	.	1.58	.	*	F	2.60	2.57
Pro	164	T	T	.	1.79	.	*	F	2.82	0.82
Asp	165	T	T	.	2.09	.	*	F	2.79	0.99
Gly	166	T	T	.	1.79	.	*	F	3.10	0.81
Cys	167	T	.	.	1.79	.	*	F	2.29	0.70
Asn	168	T	.	.	1.39	.	*	F	1.98	0.56
Asn	169	T	T	.	0.71	.	.	F	0.97	0.60
Ser	170	T	T	.	0.50	*	.	F	0.66	0.78
Ser	171	T	T	.	0.96	*	*	F	0.35	0.75
Trp	172	.	.	B	.	.	T	.	1.28	*	*	F	0.25	0.92
Ile	173	.	.	B	.	.	T	.	1.28	*	*	F	0.25	0.68
Pro	174	.	.	B	.	.	T	.	1.03	.	*	F	0.25	0.81
Arg	175	T	T	.	0.44	.	.	F	0.50	1.21
Gly	176	T	C	0.74	.	.	F	0.60	1.21
Asn	177	C	0.73	*	.	F	1.00	1.36
Tyr	178	C	1.62	*	.	.	1.04	0.93
Ile	179	.	.	B	1.94	.	*	F	0.88	1.51
Glu	180	.	.	B	1.83	.	*	F	1.82	1.84
Ser	181	.	.	B	2.18	.	.	F	2.46	1.96
Asn	182	T	T	.	1.51	.	.	F	3.40	4.67
Arg	183	T	T	.	1.44	.	*	F	3.06	1.45
Asp	184	T	T	.	1.48	.	*	F	2.72	1.56
Asp	185	T	T	.	1.18	.	*	F	2.23	0.72
Cys	186	.	.	B	B	.	.	.	0.67	.	*	.	0.94	0.49
Thr	187	.	.	B	B	.	.	.	-0.22	.	*	.	0.30	0.24
Val	188	.	.	B	B	.	.	.	-0.58	.	*	.	-0.60	0.10
Ser	189	.	.	B	B	.	.	.	-1.17	*	.	.	-0.60	0.30
Leu	190	.	.	B	B	.	.	.	-2.02	*	*	.	-0.60	0.21
Ile	191	A	.	.	B	.	.	.	-1.39	.	*	.	-0.60	0.21
Tyr	192	A	.	.	B	.	.	.	-1.89	.	*	.	-0.60	0.21
Ala	193	A	.	.	B	.	.	.	-0.99	.	*	.	-0.60	0.21
Val	194	A	.	.	B	.	.	.	-0.64	.	.	.	-0.32	0.60
His	195	A	.	.	B	.	.	.	-0.13	.	.	.	0.26	0.77
Leu	196	A	.	.	B	.	.	.	0.41	.	.	.	1.29	1.02

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Lys	197	.	.	.	B	T	.	.	0.41	.	*	F	2.12	1.36
Lys	198	T	T	.	0.14	*	*	F	2.80	1.57
Ser	199	T	T	.	0.30	*	*	F	2.52	1.41
Gly	200	T	T	.	-0.37	*	*	F	1.49	0.61
Tyr	201	.	.	B	.	.	T	.	0.44	*	*	.	0.36	0.27
Val	202	.	.	B	B	.	.	.	0.16	*	*	.	-0.32	0.34
Phe	203	.	.	B	B	.	.	.	0.11	.	*	.	-0.60	0.54
Phe	204	.	.	B	B	.	.	.	0.17	.	*	.	-0.60	0.60
Glu	205	.	.	B	B	.	.	.	-0.34	.	.	.	-0.45	1.27
Tyr	206	.	.	B	B	.	.	.	-0.10	.	.	.	-0.45	1.08
Gln	207	.	.	B	B	.	.	.	0.76	.	.	.	-0.15	2.09
Tyr	208	.	.	.	B	T	.	.	1.46	.	.	.	0.85	1.94
Val	209	.	.	.	B	T	.	.	1.27	.	.	.	0.25	1.99
Asp	210	T	T	.	0.57	.	.	F	0.65	0.81
Asn	211	T	C	0.11	.	*	F	0.15	0.45
Asn	212	T	C	0.11	.	*	.	0.00	0.52
Ile	213	.	.	B	.	.	T	.	-0.34	.	*	.	0.10	0.54
Phe	214	.	A	B	-0.19	.	*	.	-0.60	0.29
Phe	215	.	A	B	-1.08	.	*	.	-0.60	0.16
Glu	216	A	A	-1.08	.	*	.	-0.60	0.16
Phe	217	A	A	B	-1.08	.	*	.	-0.60	0.31
Phe	218	A	A	-0.19	.	*	.	-0.60	0.58
Ile	219	.	A	.	.	T	.	.	0.51	*	.	.	0.44	0.56
Gln	220	.	A	.	.	T	.	.	0.54	.	*	.	0.63	1.12
Asn	221	.	A	.	.	T	.	.	0.54	.	.	F	1.27	0.69
Asp	222	T	T	.	1.24	.	.	F	2.76	1.71
Gln	223	T	T	.	1.34	.	.	F	3.40	1.71
Cys	224	T	T	.	2.23	.	.	F	3.06	1.05
Gln	225	T	T	.	1.92	.	.	F	2.72	1.05
Glu	226	.	A	B	1.61	.	.	F	1.43	0.88
Met	227	.	A	B	1.30	*	.	F	0.94	2.37
Asp	228	.	A	B	1.30	*	.	F	0.90	1.97
Thr	229	A	A	2.01	*	.	F	0.90	1.90
Thr	230	A	T	.	1.72	*	.	F	1.30	3.84
Thr	231	A	T	.	0.87	*	.	F	1.30	2.42
Asp	232	A	T	.	1.51	*	*	F	0.40	1.24
Lys	233	A	T	.	0.70	*	.	F	1.00	1.72
Trp	234	A	A	.	B	.	.	.	0.70	*	.	.	0.30	0.99
Val	235	.	A	B	B	.	.	.	1.01	*	.	.	0.60	0.85
Lys	236	.	A	B	B	.	.	.	1.32	*	*	.	0.90	0.71
Leu	237	.	A	B	0.98	*	*	F	0.90	1.09
Thr	238	T	C	0.93	*	*	F	2.40	1.45
Asp	239	T	C	0.93	*	*	F	3.00	1.26
Asn	240	T	C	1.44	.	*	F	2.40	1.60
Gly	241	T	C	1.10	*	*	F	2.10	1.10
Glu	242	T	.	.	1.88	*	.	F	1.65	0.88
Trp	243	C	1.89	*	.	F	0.55	0.75
Gly	244	A	T	.	1.03	*	.	F	0.40	1.01
Ser	245	A	T	.	0.43	.	.	.	0.10	0.43

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
His	246	A	T	.	-0.03	.	*	.	-0.20	0.41
Ser	247	.	.	B	.	.	T	.	0.01	.	*	.	-0.20	0.34
Val	248	.	.	B	0.00	.	.	.	-0.10	0.51
Met	249	.	.	B	0.00	.	*	.	-0.10	0.50
Leu	250	.	.	B	.	.	T	.	-0.01	.	*	.	0.10	0.37
Lys	251	.	.	B	.	.	T	.	0.02	*	*	F	0.25	0.72
Ser	252	T	C	-0.57	*	*	F	0.60	1.16
Gly	253	T	C	-0.52	*	*	F	0.45	0.99
Thr	254	.	.	B	B	.	.	.	-0.17	*	.	F	-0.15	0.41
Asn	255	.	.	B	B	.	.	.	0.36	.	*	F	-0.45	0.48
Ile	256	.	.	B	B	.	.	.	0.42	.	*	.	-0.60	0.51
Leu	257	.	.	B	B	.	.	.	0.41	.	*	.	-0.60	0.69
Tyr	258	.	.	B	B	.	.	.	0.44	.	*	.	-0.60	0.62
Trp	259	.	.	B	B	.	.	.	0.41	.	.	.	-0.45	1.27
Arg	260	.	.	B	B	.	.	.	-0.48	.	*	.	-0.45	1.52
Thr	261	.	.	B	B	.	.	.	-0.40	.	*	F	-0.45	0.68
Thr	262	.	.	B	B	.	.	.	-0.19	.	*	F	-0.45	0.53
Gly	263	.	.	B	B	.	.	.	-0.29	.	*	.	-0.30	0.27
Ile	264	.	.	B	B	.	.	.	-0.30	.	*	.	-0.60	0.19
Leu	265	.	.	B	.	.	T	.	-0.37	.	*	.	-0.20	0.17
Met	266	.	.	B	.	.	T	.	-0.64	*	.	.	0.10	0.35
Gly	267	.	.	B	.	.	T	.	-1.19	*	.	F	0.25	0.50
Ser	268	A	T	.	-0.80	*	.	F	0.25	0.45
Lys	269	A	-0.12	*	.	F	0.65	0.91
Ala	270	A	-0.17	*	.	F	1.10	1.42
Val	271	A	.	.	B	.	.	.	-0.38	*	.	F	0.45	0.79
Lys	272	.	.	B	B	.	.	.	-0.89	*	.	F	0.45	0.33
Pro	273	.	.	B	B	.	.	.	-0.54	*	.	.	-0.60	0.24
Val	274	.	.	B	B	.	.	.	-0.59	*	.	.	-0.30	0.64
Leu	275	A	.	.	B	.	.	.	-0.89	.	.	.	0.30	0.52
Val	276	.	.	B	B	.	.	.	-0.34	.	.	.	-0.60	0.23
Lys	277	.	.	B	B	.	.	.	-1.28	.	*	.	-0.60	0.46
Asn	278	.	.	B	B	.	.	.	-1.07	.	*	.	-0.60	0.39
Ile	279	.	.	B	B	.	.	.	-0.56	.	.	.	0.30	0.91
Thr	280	.	.	B	B	.	.	.	-0.60	.	*	.	0.30	0.45
Ile	281	.	.	B	B	.	.	.	-0.33	.	.	.	-0.30	0.21
Glu	282	.	.	B	B	.	.	.	-0.62	.	*	.	-0.60	0.30
Gly	283	.	.	B	B	.	.	.	-0.93	.	*	.	-0.60	0.32
Val	284	.	.	B	B	.	.	.	-0.34	.	*	.	-0.60	0.67
Ala	285	.	.	B	B	.	.	.	-0.03	.	.	.	-0.30	0.52
Tyr	286	.	.	.	B	T	.	.	0.19	*	.	.	0.10	0.90
Thr	287	T	T	.	-0.51	*	.	F	0.65	0.65
Ser	288	T	T	.	-0.38	.	.	F	0.35	0.56
Glu	289	T	T	.	-0.19	.	.	F	0.35	0.55
Cys	290	.	.	B	.	.	T	.	0.44	.	.	.	0.10	0.20
Phe	291	.	.	B	0.48	.	.	.	0.50	0.31
Pro	292	T	.	.	0.44	.	.	.	0.90	0.27
Cys	293	T	.	.	0.43	.	.	.	0.30	0.50
Lys	294	.	.	B	.	.	T	.	-0.27	.	.	F	0.25	0.84

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Pro	295	T	T	.	0.10	.	.	F	0.65	0.47
Gly	296	T	T	.	0.80	*	*	F	0.80	1.17
Thr	297	T	T	.	1.06	*	.	F	1.25	0.94
Phe	298	.	.	B	1.51	*	.	F	1.08	1.22
Ser	299	.	.	B	1.12	*	.	F	1.36	1.91
Asn	300	T	.	.	1.03	.	.	F	2.04	1.31
Lys	301	T	C	0.68	.	*	F	2.32	2.03
Pro	302	T	T	.	0.99	.	.	F	2.80	1.31
Gly	303	T	T	.	1.02	.	*	F	2.52	1.31
Ser	304	T	T	.	1.32	.	*	F	1.49	0.35
Phe	305	T	.	.	0.47	*	*	.	0.56	0.39
Asn	306	.	.	B	-0.24	.	*	.	-0.12	0.29
Cys	307	.	.	B	-0.24	.	*	.	-0.40	0.12
Gln	308	.	.	B	0.21	.	*	.	-0.40	0.21
Val	309	.	.	B	0.51	.	*	.	-0.10	0.26
Cys	310	.	.	B	.	.	T	.	0.90	.	*	.	0.10	0.77
Pro	311	T	T	.	0.66	*	*	F	1.59	0.64
Arg	312	T	T	.	1.02	.	*	F	1.48	1.35
Asn	313	T	T	.	1.02	.	*	F	1.82	3.38
Thr	314	T	.	.	1.92	.	*	F	2.86	3.79
Tyr	315	T	T	.	2.24	.	.	F	3.40	3.87
Ser	316	T	C	1.87	.	*	F	2.86	2.38
Glu	317	A	.	.	.	T	T	.	1.80	.	.	F	2.42	1.67
Lys	318	A	T	.	1.80	*	.	F	1.98	2.13
Gly	319	A	A	1.44	*	.	F	1.24	2.75
Ala	320	A	A	0.80	*	*	F	0.75	0.85
Lys	321	A	A	1.21	*	*	F	0.75	0.30
Glu	322	A	A	0.54	*	*	.	0.60	0.59
Cys	323	A	A	0.54	*	*	.	0.94	0.31
Ile	324	A	A	0.89	*	*	.	1.28	0.31
Arg	325	A	A	1.48	*	*	.	1.62	0.30
Cys	326	.	.	B	.	.	T	.	1.13	*	*	.	2.36	0.94
Lys	327	T	T	.	1.13	.	*	F	3.40	1.80
Asp	328	T	T	.	1.10	.	*	F	3.06	1.59
Asp	329	T	T	.	1.69	.	*	F	2.72	2.57
Ser	330	T	.	.	1.23	.	*	F	2.18	1.72
Gln	331	T	.	.	1.60	.	*	F	1.84	1.02
Phe	332	T	T	.	1.26	.	*	F	1.25	0.82
Ser	333	T	C	1.26	.	*	F	0.45	0.82
Gly	334	T	T	.	0.59	.	*	F	1.55	0.82
Ser	335	T	C	0.58	.	*	F	1.65	0.51
Ser	336	C	0.58	*	*	F	1.75	0.55
Glu	337	T	.	.	1.39	*	*	F	2.55	0.96
Cys	338	T	.	.	1.48	*	*	F	3.00	1.40
Thr	339	T	.	.	1.61	.	*	F	2.70	1.61
Glu	340	T	.	.	1.24	*	*	F	2.74	1.44
Arg	341	C	1.23	*	*	F	2.58	1.44
Pro	342	T	C	0.92	*	*	F	2.82	1.44
Pro	343	T	T	.	1.63	*	*	F	3.06	1.20

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Cys	344	T	T	.	1.94	*	.	F	3.40	1.22
Thr	345	T	T	.	1.70	*	*	F	3.06	1.32
Thr	346	T	T	.	0.89	*	*	F	2.42	1.34
Lys	347	T	T	.	1.10	*	*	F	1.48	2.16
Asp	348	T	T	.	0.42	.	*	F	1.74	2.60
Tyr	349	.	.	B	.	.	T	.	1.06	.	*	.	0.25	1.26
Phe	350	.	.	B	B	.	.	.	1.06	*	*	.	-0.30	0.86
Gln	351	.	.	B	B	.	.	.	1.16	*	*	.	-0.60	0.74
Ile	352	.	.	B	B	.	.	.	0.44	*	*	.	-0.26	0.73
His	353	.	.	B	B	.	.	.	0.44	.	*	.	0.08	0.45
Thr	354	T	C	0.69	.	*	.	1.32	0.44
Pro	355	T	C	1.39	.	*	F	2.56	1.08
Cys	356	T	T	.	1.04	*	*	F	3.40	1.37
Asp	357	T	T	.	1.98	*	*	F	2.91	0.94
Glu	358	A	1.70	.	*	F	2.12	1.22
Glu	359	A	2.01	.	*	F	1.78	3.28
Gly	360	A	1.33	.	*	F	1.44	3.40
Lys	361	A	.	.	B	.	.	.	1.40	.	*	F	0.90	1.38
Thr	362	A	.	.	B	.	.	.	1.16	.	*	F	0.45	0.79
Gln	363	A	.	.	B	.	.	.	1.20	.	*	.	-0.15	1.25
Ile	364	A	.	.	B	.	.	.	0.91	*	*	.	0.45	1.25
Met	365	A	.	.	B	.	.	.	0.37	*	*	.	-0.60	0.91
Tyr	366	.	.	B	B	.	.	.	0.32	*	*	.	-0.60	0.37
Lys	367	.	.	B	B	.	.	.	0.42	*	*	.	-0.60	0.91
Trp	368	.	.	.	B	T	.	.	0.47	*	*	.	-0.05	1.42
Ile	369	A	.	.	B	.	.	.	0.47	.	*	.	0.45	1.81
Glu	370	A	.	.	B	.	.	.	0.40	*	.	.	0.30	0.63
Pro	371	A	A	0.76	*	.	.	-0.30	0.32
Lys	372	.	A	.	.	T	.	.	0.71	*	.	.	1.00	0.90
Ile	373	A	A	1.00	.	*	.	0.60	0.90
Cys	374	A	A	1.08	*	*	.	0.60	0.98
Arg	375	A	A	0.77	*	.	F	0.75	0.40
Glu	376	A	A	0.98	*	.	F	0.75	0.83
Asp	377	A	A	0.34	*	.	F	0.90	2.58
Leu	378	A	A	0.34	*	*	F	0.90	1.33
Thr	379	A	A	1.12	*	*	F	0.75	0.54
Asp	380	A	A	0.20	*	*	F	0.75	0.63
Ala	381	.	A	B	-0.01	*	*	.	-0.30	0.63
Ile	382	.	A	B	-0.22	*	*	.	0.30	0.68
Arg	383	.	.	B	0.29	*	*	.	0.84	0.63
Leu	384	.	.	B	0.26	*	*	.	0.58	0.83
Pro	385	T	C	0.26	*	*	F	1.62	1.17
Pro	386	T	T	.	0.89	*	*	F	3.06	1.04
Ser	387	T	T	.	1.82	*	*	F	3.40	2.52
Gly	388	T	T	.	1.71	*	*	F	3.06	3.26
Glu	389	T	.	.	1.86	.	.	F	2.75	3.52
Lys	390	T	T	.	1.86	.	.	F	2.84	1.41
Lys	391	T	T	.	1.86	.	.	F	2.73	2.20
Asp	392	T	T	.	1.49	.	.	F	2.62	1.96

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Cys	393	.	.	B	.	.	T	.	1.83	.	.	F	2.30	0.53
Pro	394	.	.	B	1.62	.	.	F	1.87	0.42
Pro	395	T	.	.	1.23	*	.	F	1.74	0.39
Cys	396	T	.	.	0.49	.	.	F	0.91	0.72
Asn	397	.	.	B	.	.	T	.	0.24	.	.	F	0.18	0.40
Pro	398	T	T	.	0.91	.	.	F	0.35	0.41
Gly	399	T	T	.	1.12	.	.	.	0.35	1.23
Phe	400	.	.	B	.	.	T	.	0.99	.	.	.	-0.05	1.23
Tyr	401	.	.	B	1.36	.	.	.	-0.40	0.79
Asn	402	T	T	.	1.06	.	.	F	0.63	1.07
Asn	403	T	T	.	0.97	.	.	F	0.76	1.65
Gly	404	T	T	.	0.64	*	.	F	1.19	1.41
Ser	405	T	T	.	1.31	*	.	F	1.77	0.47
Ser	406	T	T	.	1.34	.	.	F	1.30	0.40
Ser	407	T	T	.	0.68	.	.	F	1.17	0.62
Cys	408	.	.	B	.	.	T	.	0.47	.	.	.	0.49	0.25
His	409	.	.	B	.	.	T	.	0.60	.	.	.	0.36	0.29
Pro	410	T	.	.	0.56	.	.	.	0.43	0.33
Cys	411	.	.	B	0.54	.	.	.	-0.10	0.61
Pro	412	.	.	B	.	.	T	.	0.14	.	.	F	0.25	0.65
Pro	413	T	T	.	0.51	.	.	F	0.35	0.36
Gly	414	T	T	.	0.54	.	.	F	0.35	0.91
Thr	415	.	.	B	.	.	T	.	0.41	.	.	F	1.19	0.98
Phe	416	.	.	B	.	.	T	.	0.77	*	.	F	1.53	0.63
Ser	417	.	.	B	.	.	T	.	1.02	*	.	F	1.27	0.92
Asp	418	T	T	.	1.23	*	.	F	2.76	1.27
Gly	419	T	T	.	0.91	*	*	F	3.40	2.54
Thr	420	T	.	.	1.33	*	*	F	2.86	1.02
Lys	421	T	.	.	1.82	*	*	F	2.52	1.19
Glu	422	T	.	.	1.46	*	*	F	2.18	1.86
Cys	423	T	.	.	1.24	*	*	F	1.69	0.69
Arg	424	.	.	B	1.00	*	.	F	0.95	0.53
Pro	425	.	.	B	0.97	*	.	F	0.93	0.31
Cys	426	T	T	.	0.61	*	.	F	1.81	0.58
Pro	427	T	T	.	0.61	*	*	F	2.09	0.42
Ala	428	T	T	.	1.07	*	.	F	2.37	0.47
Gly	429	T	T	.	0.37	*	*	F	2.80	1.37
Thr	430	.	.	B	-0.23	.	.	F	1.77	0.89
Glu	431	.	.	B	0.09	.	.	F	0.89	0.73
Pro	432	A	-0.40	.	.	F	1.21	0.73
Ala	433	A	0.19	.	*	.	0.18	0.44
Leu	434	A	0.29	.	*	.	0.50	0.44
Gly	435	A	0.64	.	*	.	-0.40	0.44
Phe	436	A	0.36	.	*	.	-0.10	0.88
Glu	437	A	0.28	.	*	.	-0.25	1.12
Tyr	438	A	0.87	.	*	.	-0.25	1.19
Lys	439	T	.	.	0.82	*	*	.	0.15	2.21
Trp	440	.	.	.	B	T	.	.	0.36	*	.	.	-0.20	0.95
Trp	441	.	.	B	B	.	.	.	0.84	*	*	.	-0.60	0.50

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Asn	442	.	.	B	B	.	.	.	0.50	.	.	.	-0.60	0.39
Val	443	.	.	.	B	.	.	C	0.74	*	.	.	-0.40	0.36
Leu	444	T	C	0.10	*	*	.	0.00	0.56
Pro	445	T	T	.	0.43	*	*	F	0.52	0.34
Gly	446	T	T	.	0.41	*	*	F	0.99	0.92
Asn	447	T	T	.	0.11	.	*	F	1.31	1.61
Met	448	T	.	.	0.30	.	*	F	1.88	1.40
Lys	449	.	.	B	.	.	T	.	0.41	.	*	F	1.70	0.76
Thr	450	.	.	B	.	.	T	.	0.62	.	*	F	0.93	0.41
Ser	451	.	.	B	.	.	T	.	0.11	.	*	.	0.61	0.66
Cys	452	.	.	B	.	.	T	.	-0.23	*	.	.	0.44	0.25
Phe	453	.	.	B	B	.	.	.	0.37	.	.	.	-0.43	0.17
Asn	454	.	.	B	B	.	.	.	0.02	.	.	.	-0.29	0.20
Val	455	.	.	.	B	T	.	.	0.38	.	.	.	0.42	0.51
Gly	456	T	.	.	0.01	.	.	F	1.53	1.17
Asn	457	T	T	.	0.68	.	*	F	2.49	0.39
Ser	458	T	T	.	1.03	.	*	F	3.10	0.88
Lys	459	T	T	.	0.43	*	.	F	2.79	0.88
Cys	460	T	T	.	1.29	.	.	F	2.48	0.54
Asp	461	T	.	.	1.29	.	*	F	1.97	0.65
Gly	462	T	T	.	1.00	.	*	F	1.86	0.32
Met	463	T	C	1.30	.	*	F	0.45	0.63
Asn	464	T	C	0.40	.	*	.	0.90	0.65
Gly	465	T	C	0.48	*	.	.	0.00	0.49
Trp	466	A	0.13	*	.	.	-0.40	0.50
Glu	467	A	0.48	*	.	.	-0.10	0.31
Val	468	A	1.04	*	.	.	0.50	0.52
Ala	469	A	0.16	*	.	.	0.50	0.67
Gly	470	A	0.50	*	.	.	0.50	0.27
Asp	471	A	0.49	*	.	.	-0.10	0.63
His	472	.	.	B	0.14	*	.	F	0.65	0.84
Ile	473	.	.	B	0.41	*	.	F	0.65	0.84
Gln	474	.	.	B	.	.	T	.	0.66	*	.	F	0.85	0.51
Ser	475	.	.	B	.	.	T	.	0.66	*	.	F	0.25	0.37
Gly	476	T	T	.	0.36	*	.	F	0.65	0.52
Ala	477	T	C	0.39	*	.	F	1.35	0.40
Gly	478	C	1.28	.	.	F	1.45	0.50
Gly	479	C	1.28	.	.	F	1.75	0.82
Ser	480	C	1.33	.	.	F	2.50	1.35
Asp	481	T	C	0.87	.	.	F	3.00	2.14
Asn	482	.	.	B	.	.	T	.	0.57	.	.	F	2.20	1.78
Asp	483	.	.	B	.	.	T	.	0.10	.	.	F	1.75	0.93
Tyr	484	.	.	B	.	.	T	.	0.44	.	.	.	0.70	0.46
Leu	485	.	.	B	B	.	.	.	-0.07	.	.	.	-0.30	0.46
Ile	486	.	.	B	B	.	.	.	-0.10	.	*	.	-0.60	0.23
Leu	487	.	.	B	B	.	.	.	-0.99	.	*	.	-0.60	0.20
Asn	488	.	.	B	B	.	.	.	-1.20	.	*	.	-0.60	0.17
Leu	489	.	.	B	B	.	.	.	-1.30	.	*	.	-0.60	0.37
His	490	.	.	B	B	.	.	.	-1.19	*	*	.	-0.60	0.44

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Ile	491	.	.	B	B	.	.	.	-0.26	*	.	.	-0.60	0.24
Pro	492	T	.	.	0.34	*	*	.	0.00	0.58
Gly	493	T	.	.	0.13	.	*	F	0.45	0.66
Phe	494	T	.	.	0.63	.	.	F	0.88	1.45
Lys	495	C	0.37	.	.	F	1.56	1.36
Pro	496	T	C	0.66	.	.	F	2.04	1.84
Pro	497	T	C	0.56	.	*	F	1.72	2.10
Thr	498	T	T	.	0.56	.	*	F	2.80	1.51
Ser	499	.	.	B	.	.	T	.	0.67	.	*	F	1.37	0.97
Met	500	.	.	B	0.31	.	.	F	0.89	0.63
Thr	501	.	.	B	0.18	.	.	F	0.82	0.63
Gly	502	.	.	B	0.09	.	.	F	0.75	0.47
Ala	503	T	C	0.40	.	.	F	1.08	0.63
Thr	504	T	C	-0.11	.	.	F	1.89	0.76
Gly	505	T	C	0.14	*	.	F	2.10	0.63
Ser	506	T	C	0.57	*	*	F	1.89	0.62
Glu	507	.	.	B	0.02	*	*	F	1.58	0.84
Leu	508	.	.	B	B	.	.	.	0.30	*	*	F	0.87	0.60
Gly	509	.	.	B	B	.	.	.	-0.09	*	*	F	0.66	0.64
Arg	510	.	.	B	B	.	.	.	-0.60	*	*	.	-0.30	0.32
Ile	511	.	.	B	B	.	.	.	-1.00	*	*	.	-0.60	0.29
Thr	512	.	.	B	B	.	.	.	-1.00	*	*	.	-0.60	0.25
Phe	513	.	.	B	B	.	.	.	-0.50	*	*	.	-0.30	0.22
Val	514	.	.	B	B	.	.	.	-0.97	*	*	.	-0.60	0.46
Phe	515	.	.	B	B	.	.	.	-1.74	*	*	.	-0.60	0.26
Glu	516	.	.	B	B	.	.	.	-1.16	*	*	.	-0.60	0.16
Thr	517	A	.	.	B	.	.	.	-1.43	.	.	.	-0.60	0.29
Leu	518	A	.	.	B	.	.	.	-0.73	.	.	.	-0.60	0.34
Cys	519	.	.	.	B	T	.	.	-0.54	*	.	.	0.70	0.33
Ser	520	A	T	.	-0.70	*	.	.	0.10	0.12
Ala	521	A	T	.	-1.51	*	.	.	0.10	0.11
Asp	522	A	T	.	-1.44	.	.	.	0.10	0.17
Cys	523	A	T	.	-1.33	.	.	.	-0.20	0.20
Val	524	A	.	.	B	.	.	.	-1.27	.	*	.	-0.60	0.17
Leu	525	.	.	B	B	.	.	.	-1.82	*	*	.	-0.60	0.10
Tyr	526	.	.	B	B	.	.	.	-1.23	*	*	.	-0.60	0.14
Phe	527	.	.	B	B	.	.	.	-2.12	*	*	.	-0.60	0.31
Met	528	.	.	B	B	.	.	.	-1.46	*	*	.	-0.60	0.27
Val	529	A	.	.	B	.	.	.	-0.49	*	*	.	-0.26	0.27
Asp	530	A	.	.	B	.	.	.	0.37	*	*	.	0.38	0.62
Ile	531	A	0.31	*	*	.	1.97	1.25
Asn	532	A	T	.	0.70	*	*	F	2.66	2.27
Arg	533	T	T	.	1.30	*	*	F	3.40	1.96
Lys	534	T	T	.	1.30	*	*	F	3.06	4.49
Ser	535	T	C	0.44	*	.	F	2.52	2.07
Thr	536	.	.	.	B	.	.	C	1.33	*	.	F	1.33	0.79
Asn	537	.	.	B	B	.	.	.	1.03	*	.	F	0.79	0.68
Val	538	.	.	B	B	.	.	.	0.63	*	.	.	0.30	0.68
Val	539	.	.	B	B	.	.	.	0.24	*	.	.	-0.60	0.50

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Glu	540	.	.	B	B	.	.	.	0.20	*	.	.	-0.60	0.30
Ser	541	T	.	.	0.20	.	.	F	0.45	0.41
Trp	542	T	T	.	0.24	.	.	F	1.25	0.79
Gly	543	T	C	1.10	.	.	F	1.95	0.91
Gly	544	T	C	2.00	.	.	F	2.40	1.18
Thr	545	T	C	2.00	.	.	F	3.00	2.24
Lys	546	A	A	1.71	.	.	F	2.10	3.93
Glu	547	A	A	1.76	.	.	F	1.80	4.01
Lys	548	A	A	1.79	.	.	F	1.50	4.35
Gln	549	A	A	2.10	*	.	F	1.20	3.14
Ala	550	A	A	1.52	*	.	.	0.45	2.47
Tyr	551	A	.	.	B	.	.	.	0.59	.	.	.	-0.30	0.87
Thr	552	A	.	.	B	.	.	.	-0.11	.	*	.	-0.60	0.35
His	553	.	.	B	B	.	.	.	-0.11	.	.	.	-0.60	0.30
Ile	554	.	.	B	B	.	.	.	-0.11	*	*	.	-0.60	0.38
Ile	555	.	.	B	B	.	.	.	-0.11	*	.	.	-0.60	0.43
Phe	556	.	.	B	B	.	.	.	-0.18	.	.	.	-0.60	0.32
Lys	557	.	.	B	B	.	.	.	-0.57	.	.	.	-0.60	0.65
Asn	558	.	.	.	B	.	.	C	-0.84	*	*	.	-0.40	0.81
Ala	559	.	.	.	B	.	.	C	-0.66	.	*	.	-0.25	1.34
Thr	560	.	.	.	B	.	.	C	-0.08	*	*	.	-0.40	0.58
Phe	561	.	.	.	B	.	.	C	0.33	*	*	.	-0.40	0.52
Thr	562	.	.	B	B	.	.	.	-0.30	.	*	.	-0.60	0.54
Phe	563	.	A	B	B	.	.	.	-1.00	.	*	.	-0.60	0.38
Thr	564	.	A	B	B	.	.	.	-0.41	*	*	.	-0.60	0.38
Trp	565	.	A	B	B	.	.	.	0.01	*	*	.	-0.60	0.46
Ala	566	A	A	.	B	.	.	.	0.40	.	*	.	-0.45	1.03
Phe	567	.	A	.	B	T	.	.	0.71	*	.	.	-0.05	1.03
Gln	568	.	A	.	B	T	.	.	1.41	*	.	.	0.29	1.58
Arg	569	.	A	.	B	T	.	.	1.38	.	.	F	1.68	2.71
Thr	570	.	.	.	B	T	.	.	1.67	*	.	F	2.02	3.09
Asn	571	T	T	.	2.26	*	.	F	2.76	3.09
Gln	572	T	T	.	2.96	*	.	F	3.40	2.64
Gly	573	T	T	.	3.07	.	.	F	2.76	2.94
Gln	574	T	T	.	3.07	.	*	F	2.98	3.58
Asp	575	C	2.68	*	.	F	2.50	4.05
Asn	576	T	C	1.79	*	.	F	2.62	3.54
Arg	577	.	.	B	.	.	T	.	1.79	*	.	F	2.34	1.43
Arg	578	.	.	B	.	.	T	.	2.13	*	.	F	2.60	1.38
Phe	579	.	.	B	.	.	T	.	1.53	*	.	.	2.19	1.43
Ile	580	.	.	B	B	.	.	.	0.68	*	.	.	1.38	0.72
Asn	581	.	.	B	B	.	.	.	0.72	*	*	.	0.22	0.27
Asp	582	.	.	B	B	.	.	.	-0.28	*	*	.	-0.04	0.63
Met	583	.	.	B	B	.	.	.	-0.63	*	*	.	-0.30	0.63
Val	584	.	.	B	B	.	.	.	-0.23	*	.	.	-0.30	0.62
Lys	585	.	.	B	B	.	.	.	-0.23	*	*	.	-0.30	0.50
Ile	586	.	.	B	B	.	.	.	-0.54	*	.	.	-0.60	0.35
Tyr	587	.	.	B	B	.	.	.	-1.13	.	.	.	-0.60	0.68
Ser	588	.	.	B	B	.	.	.	-0.84	.	.	.	-0.60	0.34

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Ile	589	.	.	B	B	.	.	.	0.01	.	.	.	-0.60	0.71
Thr	590	.	.	B	B	.	.	.	-0.62	.	.	.	-0.60	0.73
Ala	591	.	.	B	B	.	.	.	-0.59	.	.	F	-0.45	0.55
Thr	592	.	.	B	B	.	.	.	-0.34	.	*	F	-0.45	0.58
Asn	593	.	.	B	B	.	.	.	-0.39	*	.	.	-0.30	0.67
Ala	594	.	.	B	.	.	T	.	-0.36	*	.	.	0.70	0.66
Val	595	.	.	B	.	.	T	.	-0.63	*	.	.	0.10	0.34
Asp	596	.	.	B	.	.	T	.	-0.34	*	.	.	0.10	0.21
Gly	597	.	.	B	.	.	T	.	-0.33	*	.	.	0.10	0.28
Val	598	.	.	B	-1.00	*	.	.	0.50	0.51
Ala	599	.	.	B	-0.30	*	*	.	0.50	0.16
Ser	600	.	.	B	.	.	T	.	-0.03	*	*	.	0.70	0.32
Ser	601	.	.	B	.	.	T	.	-0.70	*	*	.	0.70	0.44
Cys	602	.	.	B	.	.	T	.	-0.94	.	*	.	0.70	0.23
Arg	603	.	.	B	.	.	T	.	-0.90	.	*	.	0.70	0.18
Ala	604	.	.	B	-0.66	.	*	.	-0.10	0.11
Cys	605	.	.	B	-0.66	*	*	.	-0.10	0.20
Ala	606	.	.	B	-0.36	*	*	.	-0.10	0.14
Leu	607	.	.	B	0.31	*	*	.	0.24	0.24
Gly	608	.	.	B	.	.	T	.	-0.10	.	*	F	1.53	0.76
Ser	609	.	.	B	.	T	T	.	0.14	.	.	F	2.42	1.01
Glu	610	T	T	.	0.51	.	.	F	2.76	1.21
Gln	611	T	T	.	0.80	.	.	F	3.40	1.64
Ser	612	T	.	.	0.94	.	.	F	2.86	1.64
Gly	613	T	T	.	0.43	.	.	F	2.27	0.51
Ser	614	T	T	.	0.52	.	.	F	1.33	0.22
Ser	615	T	T	.	-0.14	.	.	F	0.99	0.25
Cys	616	.	.	B	.	.	T	.	-0.36	.	.	.	-0.20	0.14
Val	617	.	.	B	-0.27	.	.	.	-0.40	0.16
Pro	618	.	.	B	-0.27	.	.	.	-0.40	0.18
Cys	619	.	.	B	0.00	.	.	.	-0.40	0.33
Pro	620	.	.	B	.	.	T	.	0.06	.	.	F	-0.05	0.61
Pro	621	T	T	.	-0.17	.	.	F	0.35	0.62
Gly	622	T	T	.	0.69	*	.	.	0.20	0.81
His	623	.	.	B	.	.	T	.	0.94	*	.	.	0.10	0.91
Tyr	624	.	A	B	1.61	*	.	.	0.45	1.17
Ile	625	.	A	B	1.51	*	.	.	0.75	2.06
Glu	626	A	A	1.72	*	.	.	0.75	2.18
Lys	627	A	A	2.07	*	.	F	1.24	2.24
Glu	628	A	A	1.43	*	.	F	1.58	5.53
Thr	629	A	A	1.72	*	.	F	1.92	1.71
Asn	630	T	T	.	2.61	*	.	F	3.06	1.71
Gln	631	T	T	.	1.94	*	.	F	3.40	1.71
Cys	632	T	T	.	1.69	*	.	F	2.91	0.64
Lys	633	T	T	.	1.48	.	.	F	2.85	0.61
Glu	634	T	.	.	1.79	*	.	F	2.59	0.55
Cys	635	.	.	B	1.48	*	.	F	2.28	1.70
Pro	636	.	.	B	.	.	T	.	1.23	.	.	F	2.42	1.23
Pro	637	T	T	.	1.09	.	.	F	2.80	1.11

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Asp	638	T	T	.	0.74	.	*	F	1.92	1.71
Thr	639	A	T	.	-0.14	.	*	F	1.24	1.48
Tyr	640	.	.	B	B	.	.	.	0.49	.	*	.	-0.04	0.67
Leu	641	.	.	B	B	.	.	.	0.70	*	.	.	-0.32	0.55
Ser	642	.	.	B	B	.	.	.	0.06	*	.	.	-0.60	0.66
Ile	643	.	.	B	B	.	.	.	-0.19	.	.	.	-0.60	0.31
His	644	.	.	B	B	.	.	.	-0.22	*	.	.	-0.60	0.59
Gln	645	.	.	B	B	.	.	.	0.07	.	.	.	-0.60	0.44
Val	646	.	.	B	B	.	.	.	0.88	*	.	.	-0.45	1.24
Tyr	647	.	A	.	.	T	.	.	0.59	*	.	.	0.85	1.58
Gly	648	.	A	.	.	T	.	.	0.81	*	.	.	0.70	0.92
Lys	649	.	A	.	.	T	.	.	-0.04	.	.	F	0.85	0.67
Glu	650	.	A	.	.	T	.	.	-0.26	.	*	F	0.85	0.30
Ala	651	.	A	B	B	.	.	.	-0.07	.	.	.	0.30	0.47
Cys	652	.	A	B	B	.	.	.	-0.17	.	.	.	0.30	0.12
Ile	653	.	.	B	B	.	.	.	-0.03	.	.	.	-0.30	0.07
Pro	654	.	.	B	-0.42	.	.	.	-0.40	0.11
Cys	655	T	.	.	-0.72	.	.	.	0.00	0.20
Gly	656	T	C	-0.09	.	.	F	0.45	0.39
Pro	657	T	T	.	0.58	.	.	F	1.25	0.50
Gly	658	T	T	.	1.47	.	.	F	1.74	1.50
Ser	659	T	C	1.68	.	.	F	2.18	2.43
Lys	660	T	.	.	2.34	.	.	F	2.52	2.72
Asn	661	T	.	.	2.66	.	.	F	2.86	4.60
Asn	662	T	T	.	2.57	.	.	F	3.40	4.67
Gln	663	T	T	.	2.06	.	.	F	3.06	3.13
Asp	664	T	T	.	1.69	.	.	F	2.42	1.44
His	665	.	.	B	.	.	T	.	1.40	.	.	F	1.53	0.48
Ser	666	.	.	B	B	.	.	.	1.10	.	.	.	0.04	0.44
Val	667	.	.	B	B	.	.	.	1.10	.	.	.	-0.30	0.35
Cys	668	.	.	B	B	.	.	.	0.43	.	.	.	-0.30	0.43
Tyr	669	T	T	.	-0.27	.	.	.	0.50	0.17
Ser	670	T	T	.	-0.93	.	.	.	0.20	0.20
Asp	671	T	T	.	-0.88	.	.	.	0.20	0.32
Cys	672	.	.	B	.	.	T	.	-0.06	.	.	.	-0.20	0.32
Phe	673	.	A	B	B	.	.	.	0.61	.	.	.	-0.60	0.33
Phe	674	A	A	.	B	.	.	.	0.90	.	.	.	-0.60	0.34
Tyr	675	A	A	.	B	.	.	.	1.20	.	.	.	-0.45	1.27
His	676	A	A	.	B	.	.	.	1.20	.	.	.	0.45	2.54
Glu	677	A	A	1.87	.	.	F	0.90	4.71
Lys	678	A	A	1.68	.	.	F	0.90	5.21
Glu	679	A	A	1.57	.	.	F	0.90	2.68
Asn	680	A	A	.	B	.	.	.	1.78	*	.	F	0.90	1.28
Gln	681	A	A	.	B	.	.	.	1.57	.	.	F	0.45	0.87
Ile	682	A	A	.	B	.	.	.	1.57	.	.	.	-0.60	0.79
Leu	683	.	A	B	B	.	.	.	0.82	.	*	.	-0.60	0.82
His	684	.	A	B	B	.	.	.	0.52	.	.	.	-0.60	0.41
Tyr	685	.	.	B	B	.	.	.	0.52	*	.	.	-0.60	0.78
Asp	686	.	.	B	B	.	.	.	-0.29	*	.	.	-0.45	1.52

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Phe	687	.	.	B	.	.	T	.	0.30	*	.	.	-0.20	0.92
Ser	688	T	T	.	0.81	*	*	.	0.20	0.79
Asn	689	T	C	-0.01	*	.	F	0.45	0.63
Leu	690	.	.	B	.	.	T	.	-0.11	*	.	F	-0.05	0.54
Ser	691	C	-0.41	.	.	F	0.25	0.40
Ser	692	T	C	-0.52	*	.	F	0.45	0.33
Val	693	.	.	B	.	.	T	.	-0.82	*	.	F	-0.05	0.33
Gly	694	.	.	B	.	.	T	.	-0.82	*	.	F	-0.05	0.25
Ser	695	.	.	B	.	.	T	.	-0.36	*	.	.	-0.20	0.30
Leu	696	.	.	B	-0.27	*	.	.	-0.40	0.39
Met	697	.	.	B	-0.27	.	.	.	-0.10	0.62
Asn	698	.	.	B	-0.11	.	.	F	0.05	0.62
Gly	699	T	C	-0.08	.	.	F	0.15	0.65
Pro	700	T	C	-0.08	*	.	F	0.15	0.94
Ser	701	T	C	0.78	*	.	F	0.73	0.79
Phe	702	.	.	B	.	.	T	.	1.03	.	*	F	1.56	1.59
Thr	703	.	.	B	0.72	*	.	F	1.64	1.02
Ser	704	.	.	B	.	.	T	.	1.11	*	.	F	2.12	1.10
Lys	705	T	T	.	1.08	*	.	F	2.80	2.53
Gly	706	T	T	.	0.68	*	*	F	2.52	2.75
Thr	707	T	T	.	1.34	*	*	F	2.24	1.78
Lys	708	.	.	B	B	.	.	.	0.96	*	.	F	1.16	1.21
Tyr	709	.	.	B	B	.	.	.	0.56	*	.	.	-0.17	1.06
Phe	710	.	.	B	B	.	.	.	0.51	.	*	.	-0.60	0.63
His	711	.	.	B	B	.	.	.	-0.03	.	.	.	-0.60	0.51
Phe	712	.	.	B	B	.	.	.	-0.02	*	*	.	-0.60	0.23
Phe	713	.	.	B	B	.	.	.	-0.88	*	*	.	-0.60	0.35
Asn	714	.	.	.	B	T	.	.	-1.30	.	*	.	-0.20	0.21
Ile	715	.	.	.	B	T	.	.	-0.94	.	*	.	-0.20	0.13
Ser	716	.	.	.	B	.	.	C	-0.94	.	*	.	-0.40	0.15
Leu	717	A	.	.	B	.	.	.	-0.24	.	*	.	-0.60	0.13
Cys	718	A	T	.	0.11	.	*	.	0.10	0.32
Gly	719	A	T	.	0.16	*	*	.	0.10	0.23
His	720	A	T	.	1.09	*	.	.	0.70	0.57
Glu	721	A	T	.	0.79	*	.	F	1.30	2.12
Gly	722	A	A	1.01	.	.	F	0.90	2.12
Lys	723	A	A	0.87	*	.	F	0.90	1.57
Lys	724	A	A	0.54	*	.	F	0.75	0.75
Met	725	A	A	0.27	.	.	.	0.30	0.41
Ala	726	A	A	0.27	*	.	.	0.30	0.29
Leu	727	A	A	0.61	*	.	.	-0.30	0.24
Cys	728	.	.	B	.	.	T	.	-0.32	*	.	.	-0.20	0.38
Thr	729	.	.	B	.	.	T	.	-0.68	*	.	.	-0.20	0.27
Asn	730	.	.	B	.	.	T	.	-0.08	*	.	F	-0.05	0.46
Asn	731	T	T	.	-0.19	*	.	F	0.80	1.45
Ile	732	.	.	B	B	.	.	.	0.31	.	.	F	-0.15	0.87
Thr	733	.	.	B	B	.	.	.	0.12	*	*	F	-0.15	0.78
Asp	734	.	.	B	B	.	.	.	0.48	*	*	F	-0.45	0.36
Phe	735	.	.	B	B	.	.	.	0.48	*	.	.	-0.15	1.02

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Thr	736	.	.	B	B	.	.	.	-0.41	*	.	.	0.75	1.23
Val	737	.	.	B	B	.	.	.	-0.38	.	.	.	0.30	0.52
Lys	738	.	.	B	B	.	.	.	-0.66	.	*	.	-0.30	0.44
Glu	739	.	.	B	B	.	.	.	-1.00	.	*	.	0.30	0.31
Ile	740	.	.	B	B	.	.	.	-0.60	.	*	.	0.30	0.41
Val	741	.	.	B	B	.	.	.	-0.29	*	.	.	0.30	0.28
Ala	742	.	.	B	B	.	.	.	0.57	*	.	.	0.64	0.27
Gly	743	.	.	B	B	.	.	.	0.28	*	.	F	1.13	0.64
Ser	744	T	C	-0.03	*	.	F	2.22	1.34
Asp	745	T	T	.	0.86	*	.	F	2.76	1.92
Asp	746	T	T	.	0.90	*	.	F	3.40	3.12
Tyr	747	.	.	B	.	.	T	.	0.63	*	.	F	2.36	1.92
Thr	748	.	.	B	B	.	.	.	0.63	*	.	F	1.47	0.85
Asn	749	.	.	B	B	.	.	.	0.34	*	.	.	0.08	0.50
Leu	750	.	.	B	B	.	.	.	-0.36	*	.	.	-0.26	0.33
Val	751	.	.	B	B	.	.	.	-1.21	*	.	.	-0.60	0.20
Gly	752	.	.	B	B	.	.	.	-1.63	*	.	.	-0.60	0.09
Ala	753	.	.	B	B	.	.	.	-1.32	.	.	.	-0.60	0.06
Phe	754	.	.	B	B	.	.	.	-1.62	.	.	.	-0.60	0.14
Val	755	.	.	B	B	.	.	.	-1.12	.	.	.	-0.60	0.19
Cys	756	.	.	B	.	.	T	.	-1.16	.	.	.	-0.20	0.26
Gln	757	.	.	B	.	.	T	.	-1.70	.	.	.	-0.20	0.21
Ser	758	.	.	B	.	.	T	.	-1.32	.	.	F	-0.05	0.20
Thr	759	.	.	B	.	.	T	.	-0.92	.	.	F	-0.05	0.58
Ile	760	.	.	B	-0.07	.	.	F	-0.25	0.45
Ile	761	.	.	B	.	.	T	.	0.30	.	.	F	0.59	0.58
Pro	762	.	.	B	.	.	T	.	0.34	.	*	F	0.93	0.54
Ser	763	T	T	.	0.30	*	.	F	2.42	1.55
Glu	764	T	C	-0.09	*	.	F	2.86	2.18
Ser	765	T	T	.	0.91	*	.	F	3.40	1.22
Lys	766	T	T	.	1.21	*	*	F	3.06	1.79
Gly	767	A	T	.	0.83	*	*	F	2.32	1.04
Phe	768	A	T	.	0.32	*	*	.	1.38	0.79
Arg	769	A	0.02	*	*	.	0.84	0.32
Ala	770	A	0.02	*	*	.	-0.10	0.44
Ala	771	A	-0.02	*	*	.	-0.10	0.68
Leu	772	A	T	.	0.02	*	*	.	0.70	0.60
Ser	773	A	T	.	-0.17	*	*	F	0.25	0.80
Ser	774	A	T	.	-1.17	*	*	F	-0.05	0.55
Gln	775	.	.	B	.	.	T	.	-1.39	.	.	F	-0.05	0.47
Ser	776	.	.	B	B	.	.	.	-1.39	.	.	F	-0.45	0.29
Ile	777	.	.	B	B	.	.	.	-0.58	.	.	.	-0.60	0.22
Ile	778	.	.	B	B	.	.	.	-0.59	.	.	.	-0.30	0.21
Leu	779	.	.	B	B	.	.	.	-0.99	.	.	.	-0.60	0.23
Ala	780	.	.	B	B	.	.	.	-1.88	.	.	.	-0.60	0.28
Asp	781	.	.	B	B	.	.	.	-1.92	.	.	.	-0.60	0.28
Thr	782	.	.	B	B	.	.	.	-1.89	.	.	.	-0.60	0.34
Phe	783	.	.	B	B	.	.	.	-1.31	.	*	.	-0.60	0.25
Ile	784	.	.	B	B	.	.	.	-1.36	*	*	.	-0.60	0.21

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Gly	785	.	.	B	B	.	.	.	-0.77	.	*	.	-0.60	0.11
Val	786	.	.	B	B	.	.	.	-1.08	.	*	.	-0.60	0.22
Thr	787	.	.	B	B	.	.	.	-1.08	.	*	.	-0.30	0.45
Val	788	.	.	B	B	.	.	.	-1.19	*	*	.	-0.30	0.66
Glu	789	A	.	.	B	.	.	.	-0.26	*	*	F	-0.45	0.73
Thr	790	A	.	.	B	.	.	.	0.09	*	*	F	0.60	1.01
Thr	791	A	.	.	B	.	.	.	0.06	*	*	F	0.60	2.19
Leu	792	A	.	.	B	.	.	.	0.37	*	*	F	0.45	0.89
Lys	793	A	.	.	B	.	.	.	0.33	*	*	F	-0.15	0.99
Asn	794	A	.	.	B	.	.	.	0.38	.	*	F	-0.15	0.48
Ile	795	A	.	.	B	.	.	.	0.69	.	*	F	0.60	1.17
Asn	796	A	A	.	B	.	.	.	1.00	.	*	F	0.90	1.01
Ile	797	.	A	B	B	.	.	.	1.21	*	*	F	0.90	1.05
Lys	798	.	A	B	B	.	.	.	0.47	.	*	F	0.90	1.48
Glu	799	.	A	B	0.26	.	*	F	0.75	0.80
Asp	800	.	A	B	0.29	.	*	F	0.60	1.76
Met	801	.	A	B	B	.	.	.	0.08	*	*	.	0.60	0.65
Phe	802	.	A	B	B	.	.	.	0.66	.	*	.	0.30	0.58
Pro	803	.	A	B	B	.	.	.	0.31	.	.	.	-0.30	0.50
Val	804	.	.	.	B	.	.	C	0.31	*	.	.	-0.40	0.68
Pro	805	T	T	C	-0.58	*	.	F	0.30	1.36
Thr	806	T	T	.	-0.19	*	.	F	0.35	0.62
Ser	807	T	T	C	0.51	*	.	F	0.30	1.29
Gln	808	.	.	B	.	.	T	.	-0.13	.	.	F	1.00	1.39
Ile	809	.	.	B	B	.	.	.	0.69	.	.	F	-0.15	0.71
Pro	810	.	.	B	B	.	.	.	0.20	.	.	F	-0.15	0.73
Asp	811	.	.	B	B	.	.	.	-0.19	.	.	F	-0.45	0.36
Val	812	.	.	B	B	.	.	.	-0.13	.	*	.	-0.56	0.45
His	813	.	.	B	B	.	.	.	-0.09	.	*	.	-0.52	0.45
Phe	814	.	.	B	B	.	.	.	0.50	*	*	.	-0.48	0.54
Phe	815	.	.	B	B	.	.	.	0.41	.	*	.	-0.44	0.98
Tyr	816	T	T	.	0.10	.	*	.	0.40	0.97
Lys	817	T	T	.	0.37	*	*	F	0.66	1.61
Ser	818	T	T	.	0.09	.	.	F	0.92	1.88
Ser	819	T	T	.	0.48	.	.	F	0.88	1.73
Thr	820	.	.	.	B	T	.	.	0.88	.	.	F	1.04	1.25
Ala	821	.	.	.	B	T	.	.	0.46	.	.	F	0.40	1.25
Thr	822	.	.	B	B	.	.	.	-0.48	*	.	F	-0.15	0.50
Thr	823	.	.	B	B	.	.	.	-0.18	.	.	F	-0.45	0.24
Ser	824	.	.	B	B	.	.	.	-0.22	.	*	.	-0.35	0.39
Cys	825	.	.	B	.	.	T	.	0.20	.	*	.	0.30	0.27
Ile	826	.	.	B	.	.	T	.	0.49	.	*	.	1.45	0.36
Asn	827	T	T	.	0.49	.	*	F	2.25	0.36
Gly	828	T	T	.	0.21	.	*	F	2.50	0.97
Arg	829	.	.	.	B	T	.	.	-0.34	.	*	F	2.00	1.40
Ser	830	.	.	.	B	.	.	C	0.37	.	*	F	1.40	0.64
Thr	831	.	A	B	B	.	.	.	0.66	.	*	F	1.40	1.30
Ala	832	.	A	B	B	.	.	.	0.77	.	*	F	0.70	0.66
Val	833	.	A	B	B	.	.	.	0.44	.	*	.	0.30	0.96

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Lys	834	.	A	B	B	.	.	.	0.33	.	*	.	0.30	0.36
Met	835	.	A	B	0.42	.	*	.	0.64	0.57
Arg	836	.	A	B	0.42	.	*	.	1.13	1.18
Cys	837	.	.	B	1.06	.	*	.	1.52	0.85
Asn	838	.	.	B	.	.	T	.	1.61	.	*	F	2.36	1.73
Pro	839	T	T	.	1.22	.	*	F	3.40	1.18
Thr	840	T	T	.	1.23	.	*	F	2.76	2.18
Lys	841	T	T	.	0.78	.	*	F	2.42	1.37
Ser	842	C	0.59	*	.	F	1.53	0.88
Gly	843	.	.	B	.	.	T	.	-0.30	*	.	F	1.19	0.45
Ala	844	.	.	B	.	.	T	.	-0.39	*	.	F	0.25	0.16
Gly	845	.	.	B	.	.	T	.	-0.93	*	.	.	-0.20	0.16
Val	846	.	.	B	.	.	T	.	-1.19	*	.	.	-0.20	0.12
Ile	847	.	.	B	-1.19	*	*	.	-0.40	0.18
Ser	848	.	.	B	-0.80	*	*	.	-0.15	0.25
Val	849	.	.	B	.	.	T	.	-0.88	*	*	F	0.75	0.66
Pro	850	.	.	B	.	.	T	.	-0.74	.	*	F	1.00	0.51
Ser	851	T	T	.	-0.48	*	*	F	2.25	0.58
Lys	852	T	T	.	0.07	.	*	F	2.50	0.80
Cys	853	.	.	B	.	.	T	.	0.06	.	*	F	1.85	0.51
Pro	854	T	T	.	0.24	.	*	F	2.00	0.55
Ala	855	T	T	.	0.46	.	.	F	1.75	0.15
Gly	856	T	T	.	0.41	.	.	F	1.63	0.46
Thr	857	.	.	B	-0.30	.	.	F	0.91	0.29
Cys	858	.	.	B	.	.	T	.	0.06	.	.	F	1.24	0.16
Asp	859	T	T	.	-0.43	.	.	F	1.77	0.23
Gly	860	T	T	.	-0.09	.	.	F	1.30	0.14
Cys	861	.	.	B	.	.	T	.	-0.44	.	.	.	0.32	0.40
Thr	862	.	.	B	-0.94	.	.	.	-0.01	0.21
Phe	863	.	A	B	-0.57	.	.	.	-0.34	0.17
Tyr	864	.	A	B	-0.57	.	.	.	-0.47	0.34
Phe	865	.	A	B	-0.52	.	.	.	-0.60	0.40
Leu	866	A	A	-0.44	*	.	.	-0.60	0.63
Trp	867	A	A	-0.13	.	.	.	-0.60	0.40
Glu	868	A	A	-0.02	*	.	.	-0.30	0.81
Ser	869	A	A	-0.44	.	.	.	0.30	0.99
Ala	870	A	A	0.04	.	.	.	0.30	0.50
Glu	871	A	A	0.04	.	.	.	0.60	0.45
Ala	872	A	A	-0.33	.	.	.	-0.30	0.28
Cys	873	A	T	.	-0.64	.	.	.	0.10	0.15
Pro	874	A	T	.	-0.34	.	.	.	0.10	0.12
Leu	875	A	T	.	0.21	.	.	.	0.10	0.21
Cys	876	A	T	.	0.21	.	*	.	0.10	0.53
Thr	877	A	A	0.10	.	*	.	0.30	0.58
Glu	878	A	A	0.73	.	*	.	-0.30	0.60
His	879	A	A	0.94	.	.	.	0.45	1.53
Asp	880	A	A	0.87	.	.	.	0.75	1.84
Phe	881	A	A	1.53	.	.	.	0.60	0.75
His	882	A	A	1.50	.	.	.	0.60	0.95

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Glu	883	A	A	0.91	*	.	.	0.60	0.56
Ile	884	A	A	0.28	*	*	.	0.30	0.66
Glu	885	A	A	0.32	*	.	.	0.30	0.26
Gly	886	A	A	1.13	*	.	.	0.60	0.30
Ala	887	A	A	0.82	*	.	.	0.94	0.83
Cys	888	A	T	.	0.12	*	*	.	1.68	0.48
Lys	889	A	T	.	1.01	*	.	F	1.87	0.42
Arg	890	A	.	.	.	T	T	.	1.01	*	.	F	2.61	0.71
Gly	891	T	T	.	1.04	*	.	F	3.40	2.31
Phe	892	A	.	.	B	.	.	.	0.82	*	.	F	2.26	1.67
Gln	893	.	.	B	B	.	.	.	1.24	*	.	F	1.47	0.70
Glu	894	.	.	B	B	.	.	.	0.34	*	.	F	0.38	1.11
Thr	895	.	.	B	B	.	.	.	-0.06	*	.	.	-0.26	0.95
Leu	896	.	.	B	B	.	.	.	0.29	*	.	.	-0.60	0.58
Tyr	897	.	.	B	B	.	.	.	0.99	*	.	.	-0.60	0.54
Val	898	.	.	.	B	T	.	.	0.78	.	.	.	-0.20	0.64
Trp	899	.	.	.	B	T	.	.	0.82	.	*	.	-0.05	1.21
Asn	900	.	.	.	B	.	.	C	0.84	.	*	.	0.05	1.54
Glu	901	A	.	.	B	.	.	.	0.99	.	*	F	0.00	2.18
Pro	902	T	T	.	0.34	*	*	F	0.80	1.11
Lys	903	T	T	.	1.24	*	*	F	0.65	0.49
Trp	904	T	T	.	1.19	*	*	.	1.10	0.56
Cys	905	.	.	B	.	.	T	.	0.30	*	*	.	0.10	0.36
Ile	906	.	.	B	B	.	.	.	0.00	*	*	.	-0.30	0.13
Lys	907	.	.	B	B	.	.	.	-0.60	*	*	.	-0.60	0.16
Gly	908	.	.	B	B	.	.	.	-0.86	*	*	.	-0.60	0.25
Ile	909	.	.	B	B	.	.	.	-0.57	*	*	.	-0.30	0.54
Ser	910	.	A	C	0.14	*	.	F	0.65	0.47
Leu	911	.	A	C	1.08	*	.	F	0.65	0.95
Pro	912	A	A	0.22	*	.	F	0.90	2.71
Glu	913	A	A	-0.02	*	.	F	0.90	1.67
Lys	914	A	A	0.56	*	.	F	0.90	2.05
Lys	915	A	A	0.19	.	.	F	0.90	1.91
Leu	916	A	A	1.00	.	.	F	0.75	0.59
Ala	917	A	A	0.90	.	.	.	0.60	0.51
Thr	918	A	.	.	B	.	.	.	0.04	.	.	.	0.30	0.37
Cys	919	A	.	.	B	.	.	.	0.00	.	*	.	-0.30	0.33
Glu	920	A	.	.	B	.	.	.	-0.74	.	.	.	0.30	0.55
Thr	921	A	.	.	B	.	.	.	-0.22	.	*	.	0.30	0.33
Val	922	A	.	.	B	.	.	.	-0.44	*	*	.	-0.60	0.65
Asp	923	A	.	.	B	.	.	.	-0.09	*	*	.	-0.60	0.31
Phe	924	A	.	.	B	.	.	.	-0.28	*	*	.	-0.60	0.43
Trp	925	A	.	.	B	.	.	.	-0.62	*	*	.	-0.60	0.43
Leu	926	A	.	.	B	.	.	.	-0.90	*	*	.	-0.60	0.25
Lys	927	A	.	.	B	.	.	.	-0.39	*	*	.	-0.60	0.29
Val	928	A	.	.	B	.	.	.	-1.24	*	*	.	-0.60	0.28
Gly	929	T	C	-0.89	.	*	.	0.30	0.25
Ala	930	T	C	-1.19	*	*	.	0.30	0.12
Gly	931	T	C	-1.08	.	*	.	0.00	0.17

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Val	932	.	.	B	.	.	T	.	-1.43	.	*	.	-0.20	0.15
Gly	933	.	.	B	B	.	.	.	-1.17	.	.	.	-0.60	0.21
Ala	934	.	.	B	B	.	.	.	-1.68	.	.	.	-0.60	0.21
Phe	935	.	.	B	B	.	.	.	-1.90	.	.	.	-0.60	0.21
Thr	936	A	.	.	B	.	.	.	-2.37	.	.	.	-0.60	0.18
Ala	937	A	.	.	B	.	.	.	-2.37	.	.	.	-0.60	0.15
Val	938	A	.	.	B	.	.	.	-2.61	.	.	.	-0.60	0.13
Leu	939	A	.	.	B	.	.	.	-2.83	.	.	.	-0.60	0.09
Leu	940	A	.	.	B	.	.	.	-2.44	.	.	.	-0.60	0.07
Val	941	A	.	.	B	.	.	.	-2.80	.	.	.	-0.60	0.14
Ala	942	A	.	.	B	.	.	.	-2.46	.	.	.	-0.60	0.09
Leu	943	A	.	.	B	.	.	.	-2.30	.	.	.	-0.60	0.17
Thr	944	A	.	.	B	.	.	.	-1.78	*	.	.	-0.60	0.20
Cys	945	A	.	.	B	.	.	.	-0.92	*	.	.	-0.60	0.21
Tyr	946	A	.	.	B	.	.	.	-0.02	.	.	.	-0.60	0.51
Phe	947	A	.	.	B	.	.	.	0.57	.	.	.	-0.60	0.70
Trp	948	A	.	.	B	.	.	.	1.38	*	.	.	-0.45	2.10
Lys	949	A	T	.	1.73	*	.	F	0.40	2.32
Lys	950	A	T	.	1.59	*	*	F	1.00	5.37
Asn	951	A	T	.	1.83	*	*	F	1.30	4.21
Gln	952	A	T	.	2.29	*	*	F	1.30	3.65
Lys	953	A	2.62	*	*	F	1.10	2.86
Leu	954	A	2.33	*	*	F	1.10	3.55
Glu	955	A	1.99	*	*	.	0.65	3.21
Tyr	956	A	T	.	2.03	*	*	.	0.85	2.15
Lys	957	A	T	.	1.22	*	*	F	1.00	5.22
Tyr	958	A	T	.	0.32	*	*	F	1.00	2.49
Ser	959	A	T	.	0.53	*	*	F	0.40	1.18
Lys	960	.	.	B	B	.	.	.	0.22	*	.	F	-0.15	0.58
Leu	961	.	.	B	B	.	.	.	0.16	*	.	.	-0.60	0.54
Val	962	.	.	B	B	.	.	.	0.11	*	.	.	-0.30	0.58
Met	963	.	.	B	B	.	.	.	0.06	*	*	.	0.00	0.47
Thr	964	.	.	B	.	.	T	.	0.40	.	*	F	0.55	0.76
Thr	965	A	T	.	0.36	.	*	F	1.30	2.04
Asn	966	T	C	0.50	.	.	F	2.70	3.56
Ser	967	T	C	1.36	.	.	F	3.00	1.32
Lys	968	A	A	1.14	.	.	F	2.10	1.59
Glu	969	A	A	1.24	.	.	F	1.65	0.81
Cys	970	A	A	0.97	.	.	.	1.20	0.94
Glu	971	A	A	0.38	.	.	.	0.90	0.47
Leu	972	A	A	0.68	.	.	.	0.30	0.28
Pro	973	A	A	0.33	.	.	.	0.30	0.86
Ala	974	A	A	-0.33	.	.	.	0.30	0.67
Ala	975	A	A	-0.26	.	.	.	-0.30	0.43
Asp	976	A	T	.	-1.14	.	.	.	0.70	0.28
Ser	977	A	T	.	-0.93	.	.	.	0.10	0.20
Cys	978	A	T	.	-0.72	.	.	.	0.10	0.19
Ala	979	A	T	.	-0.48	.	.	.	0.70	0.20
Ile	980	A	0.11	.	.	.	-0.10	0.15

Table II (Continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Met	981	A	0.11	.	.	.	0.50	0.48
Glu	982	A	0.41	.	.	F	0.95	0.79
Gly	983	A	T	.	1.08	.	.	F	1.30	1.81
Glu	984	A	T	.	1.67	*	.	F	1.30	3.16
Asp	985	A	T	.	2.56	*	.	F	1.30	3.16
Asn	986	A	T	.	2.30	*	.	F	1.30	5.54
Glu	987	A	A	1.44	*	.	F	0.90	2.37
Glu	988	A	A	1.54	.	.	F	0.90	1.05
Glu	989	A	A	1.24	.	.	F	0.90	1.03
Val	990	A	A	1.24	.	.	.	0.60	0.80
Val	991	A	A	1.29	.	.	.	0.30	0.74
Tyr	992	A	T	.	1.29	.	.	.	0.70	0.85
Ser	993	A	T	.	0.99	*	.	F	0.40	1.99
Asn	994	A	T	.	0.18	.	.	F	1.00	3.59
Lys	995	A	T	.	0.22	.	.	F	0.40	1.89
Gln	996	A	0.73	.	.	F	0.80	1.16
Ser	997	A	1.02	.	*	F	0.65	0.72
Leu	998	.	A	B	0.51	.	*	F	0.45	0.72
Leu	999	A	A	0.56	.	*	F	-0.15	0.34
Gly	1000	A	A	0.21	*	*	F	0.45	0.51
Lys	1001	A	A	-0.60	*	*	F	0.45	0.83
Leu	1002	A	A	-0.89	*	*	F	0.45	0.83
Lys	1003	A	A	-0.39	*	*	F	0.45	0.84
Ser	1004	A	A	0.47	*	.	F	0.45	0.61
Leu	1005	A	A	0.81	.	*	F	0.60	1.48
Ala	1006	A	A	0.81	*	*	F	0.90	1.28
Thr	1007	A	A	1.62	*	.	F	0.90	1.91
Lys	1008	A	A	1.58	.	.	F	0.90	4.01
Glu	1009	A	A	1.84	.	.	F	0.90	6.63
Lys	1010	A	A	1.96	.	.	F	0.90	6.25
Glu	1011	A	A	2.54	*	.	F	0.90	2.71
Asp	1012	A	A	2.56	*	.	F	0.90	2.71
His	1013	A	A	1.66	*	.	F	0.90	1.81
Phe	1014	A	A	1.66	.	.	.	0.60	0.78
Glu	1015	A	A	0.80	.	*	.	0.30	0.81
Ser	1016	A	A	0.84	.	*	.	-0.60	0.49
Val	1017	A	A	0.53	.	*	.	0.45	1.13
Gln	1018	A	A	0.27	*	.	.	0.30	0.94
Leu	1019	A	A	1.08	*	.	F	0.45	0.94
Lys	1020	.	A	.	.	T	.	.	0.78	*	*	F	1.00	2.48
Thr	1021	.	A	.	.	T	.	.	0.87	*	*	F	1.30	1.92
Ser	1022	.	A	.	.	T	.	.	1.72	.	*	F	1.30	3.60
Arg	1023	C	0.83	.	.	F	1.32	2.89
Ser	1024	T	C	1.26	.	.	F	1.24	1.41
Pro	1025	T	C	0.82	.	.	F	1.26	1.34
Asn	1026	T	T	.	0.74	.	.	.	1.18	0.87
Ile	1027	.	.	B	.	.	T	.	0.66	.	.	.	0.20	0.83

[0063] In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to all or a portion of a polynucleotide encoding the TR16 polypeptides described herein, including, but not limited to the TR16 polypeptides shown in Figures 1A-E and 4A-E, and encoded by one or both of the cDNA clones contained in ATCC Deposit No. PTA-506, or to the complementary strand of nucleotides 178 to 198, 298 to 321, 496 to 519, 643 to 666, 730 to 753, 838 to 861, 988 to 1011, 1072 to 1095, 1252 to 1275, 1381 to 1404, 1474 to 1497, 1576 to 1599, 1714 to 1737, 1978 to 2001, 2152 to 2175, 2341 to 2364, 2440 to 2463, 2539 to 2562, 2668 to 2691, 2848 to 2871, 500 to 1330, and/or 2500 to 2884 shown in Figure 1A-E (SEQ ID NO:1). In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to the complementary strand of nucleotides 178 to 198, 298 to 321, 496 to 519, 643 to 666, 730 to 753, 838 to 861, 988 to 1011, 1072 to 1095, 1252 to 1275, 1381 to 1404, 1474 to 1497, 1576 to 1599, 1714 to 1737, 1978 to 2001, 2152 to 2175, 2341 to 2364, 2440 to 2463, 2539 to 2562, 2668 to 2691, 2848 to 2871, 3113 to 3036, 500 to 1330, and/or 2500 to 2859 shown in Figures 4A-E (SEQ ID NO:3). By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (750 mM NaCl, 75mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C. Polypeptides encoded by these nucleic acids are also encompassed by the invention.

[0064] By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful, for example, as diagnostic probes and primers as discussed above and in more detail below. In this context "about" includes the particularly recited size, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

[0065] By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in Figures

1A-E (SEQ ID NO:1), or the nucleotide sequence as shown in Figures 4A-E (SEQ ID NO:3)).

[0066] Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the TR16 cDNA shown in Figures 1A-E (SEQ ID NO:1) and Figures 4A-E (SEQ ID NO:3)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

[0067] One skilled in the art will readily recognize thousands of individual polynucleotides that hybridize to the TR16 coding regions described herein under the stringent hybridization conditions described above. For example, and not by way of limitation, the particular polypeptide coding region shown in Figures 1A-E from nucleotide 1 to 2889 has 2889 nucleotides. Any polynucleotide having this 2889 nucleotide sequence except for one, single nucleotide substitution would hybridize to the 2889 nucleotide sequence shown in Figures 1A-E. Since each of the 2889 positions can contain any one of three substitute nucleotides, one could immediately identify $3 \times 2889 = 8667$ different embodiments of a polynucleotide that would hybridize to the coding sequence shown in Figures 1A-E. Of course, myriad other embodiments that would also hybridize to this sequence can be readily ascertained based on the nucleotide sequence provided in Figures 1A-E. These same principles can just as readily be applied to polynucleotides encoding fragments of the TR16 polypeptide shown in Figures 1A-E, as well as polynucleotides encoding all or fragments of the polypeptide shown in Figures 4A-E.

[0068] In specific embodiments, the polynucleotides of the invention are less than 110000 kb, 50000 kb, 10000 kb, 1000 kb, 500 kb, 400 kb, 350 kb, 300 kb, 250 kb, 200 kb, 175 kb, 150 kb, 125 kb, 100 kb, 75 kb, 50 kb, 40 kb, 30 kb, 25 kb, 20 kb, 15 kb, 10 kb, 7.5 kb, or 5 kb in length.

[0069] In further embodiments, polynucleotides of the invention comprise at least 15, at least 30, at least 50, at least 100, at least 250, at least 500, or at least 1000 contiguous nucleotides of TR16 coding sequence, but consist of less than or equal to 107 kb, 75 kb, 50 kb, 30 kb, 25 kb, 20 kb, 15 kb, 10 kb, or 5 kb of genomic DNA that flanks the 5' or 3'

coding nucleotide set forth in Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3). In further embodiments, polynucleotides of the invention comprise at least 15, at least 30, at least 50, at least 100, or at least 250, at least 500, at least 1000 contiguous nucleotides of TR16 and/or coding sequence, but do not comprise all or a portion of any TR16 intron. In another embodiment, the nucleic acid comprising TR16 coding sequence does not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the TR16 gene in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

[0070] As indicated, nucleic acid molecules of the present invention which encode a TR16 polypeptide may include, but are not limited to, the coding sequence for the mature polypeptide, by itself; the coding sequence for the mature polypeptide and additional sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or pre-pro-protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing - including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al.*, *Cell* 37:767-778 (1984). As discussed below, other such fusion proteins include the TR16 receptor fused to Fc at the N- or C-terminus.

[0071] The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs, or derivatives of TR16. Variants

may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

[0072] Such variants include those produced by nucleotide substitutions, deletions or additions which may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions, or additions. Especially preferred among these are silent substitutions, additions, and deletions, which do not alter the properties and activities of the TR16 receptor or portions thereof. Also especially preferred in this regard are conservative substitutions.

[0073] Further embodiments of the invention include isolated nucleic acid molecules comprising, or alternatively consisting of, a nucleotide sequence at least 80%, 85%, 90% identical, and more preferably at least 95%, 96%, 97%, 98%, or 99% identical to: (a) a nucleotide sequence encoding the polypeptide having the amino acid sequence shown in Figures 1A-E (SEQ ID NO:2); (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence shown in Figures 4A-E (SEQ ID NO:4); (c) a nucleotide sequence encoding the polypeptide having the amino acid sequence in Figures 1A-E (SEQ ID NO: 2), but lacking the amino terminal methionine; (d) a nucleotide sequence encoding a polypeptide having the amino acid sequence in Figures 4A-E (SEQ ID NO:4), but lacking the amino terminal methionine; (e) a nucleotide sequence encoding the polypeptide having the amino acid sequence at positions about 48 to about 963 in Figures 1A-E (SEQ ID NO:2); (f) a nucleotide sequence encoding a polypeptide having the amino acid sequence at positions about 48 to about 1027 in Figures 4A-E (SEQ ID NO:4); (g) a nucleotide sequence encoding a polypeptide having the amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-506; (h) a nucleotide sequence encoding a mature TR16 polypeptide having the amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-506; (i) a nucleotide sequence encoding the TR16 extracellular domain; (j) a nucleotide sequence encoding the TR16 cysteine rich domain and/or a nucleotide sequence encoding one, two, three or all four TR16 cysteine rich motifs; (k) a nucleotide sequence encoding the TR16 transmembrane domain; (l) a nucleotide sequence encoding the TR16-short intracellular domain; (m) a nucleotide

sequence encoding TR16-long intracellular domain; (n) a nucleotide sequence encoding TR16 extracellular and intracellular domains with all or part of the transmembrane domain deleted; and (o) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), or (n) above. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0074] By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a TR16 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five mismatches per each 100 nucleotides of the reference nucleotide sequence encoding the TR16 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mismatches of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The reference (query) sequence may be the entire TR16-short or TR16-long encoding nucleotide sequence shown in Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3) respectively, or any TR16-short or TR16-long polynucleotide fragment (e.g., a polynucleotide encoding the amino acid sequence of any of the TR16-short or TR16-long N- and/or C- terminal deletions described herein), variant, derivative or analog, as described herein.

[0075] As a practical matter, whether any particular nucleic acid molecule is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3) or to a nucleotide sequence of the deposited cDNA clones can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other

sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

[0076] In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. A determination of whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of this embodiment. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score. For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in

the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

[0077] The present application is directed to nucleic acid molecules comprising, or alternatively consisting of a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence, for example, shown in Figures 1A-E (SEQ ID NO:1), shown in Figures 4A-E (SEQ ID NO:3), or to the nucleic acid sequence of a deposited cDNA, irrespective of whether they encode a polypeptide having TR16-short or TR16-long receptor activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having TR16-short or TR16-long functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having TR16 receptor activity include, *inter alia*: (1) isolating the TR16 gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the TR16 gene, as described in Verma *et al.*, *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting TR16-short or TR16-long receptor mRNA expression in specific tissues.

[0078] Preferred, however, are nucleic acid molecules comprising, or alternatively consisting of, a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to for example, the nucleic acid sequence shown in Figures 1A-E (SEQ ID NO:1), or Figures 4A-E (SEQ ID NO:3), or to a nucleic acid sequence contained in one of the deposited cDNAs, which do, in fact, encode a polypeptide having TR16 functional activity. By "a polypeptide having TR16 functional activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the TR16-short

and/or TR16-long receptor of the invention (either the full-length protein or, preferably, the mature protein), as measured in a particular biological assay.

[0079] Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to, for example, a nucleic acid sequence contained in one of the deposited cDNAs or the nucleic acid sequence shown in Figures 1A-E (SEQ ID NO:1), will encode a polypeptide "having TR16 functional activity." Similarly, a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to, for example, a nucleic acid sequence contained in one of the deposited cDNAs, or the nucleic acid sequence shown in Figures 1A-E and/or Figures 4A-E, will encode a polypeptide "having TR16 functional activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing a biological assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having TR16 functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

[0080] For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in J.U. Bowie *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

POLYNUCLEOTIDE ASSAYS

[0081] This invention is also related to the use of TR16 polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of a normal and mutated form of TR16-short or TR16-long associated with a dysfunction will provide a diagnostic tool that can add or define a diagnosis of a disease or susceptibility to a disease which results from under-expression over-expression or altered expression of TR16-short or TR16-long (or a soluble form thereof), such as, for example,

tumors or autoimmune disease.

[0082] Individuals carrying mutations in the TR16 gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a biological sample from a patient (e.g., a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material). The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis. (Saiki *et al.*, *Nature* 324:163-166 (1986)). RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the nucleic acid encoding TR16-short and/or TR16-long can be used to identify and analyze TR16-short and/or TR16-long expression and mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled TR16 short and/or TR16-long RNA or alternatively, radiolabeled TR16 short and/or TR16-long antisense DNA sequences. Perfectly matched sequences can routinely be distinguished from mismatched duplexes by techniques known in the art, such as, for example, RNase A digestion or by differences in melting temperatures.

[0083] Sequence differences between a reference gene and genes having mutations also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

[0084] Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis using techniques known in the art. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al.*, *Science* 230:1242 (1985)).

[0085] Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton *et al.*, *Proc. Natl. Acad. Sci. USA* 85: 4397-4401 (1985)).

[0086] Thus, the detection of a specific DNA sequence may be achieved by methods which include, but are not limited to, hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA.

[0087] In addition to more conventional gel-electrophoresis and DNA sequencing, mutations also can be detected by *in situ* analysis.

VECTORS AND HOST CELLS

[0088] The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors and/or nucleic acids of the invention and the production of TR16 polypeptides or fragments thereof by recombinant techniques.

[0089] Host cells can be genetically engineered to incorporate nucleic acid molecules and express polypeptides of the present invention. The polynucleotides may be introduced alone or with other polynucleotides. Such other polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

[0090] In accordance with the present invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well known techniques for introducing DNA and RNA into cells. Viral vectors may be replication competent or replication defective. In the latter case viral propagation generally will occur only in complementing host cells.

[0091] Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors either are supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

[0092] The polynucleotides may be joined to a vector containing a selectable marker

for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

[0093] The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac*, *trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

[0094] As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[0095] Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

[0096] The present invention also relates to host cells containing the above-described vector constructs described herein, and additionally encompasses host cells containing nucleotide sequences of the invention that are operably associated with one or more

heterologous control regions (e.g., promoter and/or enhancer) using techniques known of in the art. The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

[0097] Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al.*, *Basic Methods In Molecular Biology* (1986).

[0098] In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., TR16 coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with TR16 polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous TR16 polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous TR16 polynucleotide sequences via homologous recombination (see, e.g., US Patent Number 5,641,670, issued June 24, 1997; International Publication Number WO 96/29411; International Publication Number WO 94/12650; Koller *et al.*, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); and Zijlstra *et al.*, *Nature* 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[0099] The TR16 polypeptide may be expressed in a modified form, such as a fusion

protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals but also additional heterologous functional regions. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. For example, in one embodiment, polynucleotides encoding TR16-short or TR16-long polypeptides of the invention may be fused to the pelB pectate lyase signal sequence to increase the efficiency to expression and purification of such polypeptides in Gram-negative bacteria. See, US Patent Nos. 5,576,195 and 5,846,818, the contents of which are herein incorporated by reference in their entireties.

[0100] A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses, it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when the Fc portion proves to be a hindrance to use in therapy and diagnosis, for example, when the fusion protein is to be used as an antigen for immunizations. In drug discovery, for example, human proteins, such as the hIL5-receptor, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *Journal of Molecular Recognition* 8:52-58 (1995) and K. Johanson *et al.*, *The Journal of Biological Chemistry* 270:16:9459-9471 (1995).

[0101] Polypeptides of the present invention include naturally purified products,

products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

[0102] In addition, proteins of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y. (1983), and Hunkapiller, *et al.*, *Nature* 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of the TR16-short and/or TR16-long polypeptides of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the TR16 polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0103] The invention additionally, encompasses TR16 polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH_4 , acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin; etc.

[0104] Additional post-translational modifications encompassed by the invention

include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

[0105] Also provided by the invention are chemically modified derivatives of TR16 which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U. S. Patent No. 4,179,337). The chemical moieties for derivation may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0106] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[0107] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*,

Nucleosides Nucleotides 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0108] The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[0109] As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

[0110] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a

population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0111] As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0112] One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($\text{ClSO}_2\text{CH}_2\text{CF}_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

[0113] Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

[0114] The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

[0115] As mentioned the TR16 proteins of the invention may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given TR16 polypeptide. TR16 polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic TR16 polypeptides may result from natural posttranslational processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter *et al.*, *Meth Enzymol* 182:626-646 (1990); Rattan *et al.*, *Ann NY Acad Sci* 663:48-62 (1992)).

[0116] The TR16 polypeptides of the invention can be recovered and purified from chemical synthesis and recombinant cell cultures by standard methods which include, but

are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

[0117] TR16 receptor polynucleotides and polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of TR16. Among these are applications in treatment of tumors, resistance to parasites, bacteria and viruses, to inhibit proliferation of B cells, to induce proliferation of T-cells, endothelial cells and certain hematopoietic cells, to treat restenosis, graft vs. host disease, to regulate anti-viral responses and to prevent certain autoimmune diseases after stimulation of TR16 by an agonist. Additional applications relate to diagnosis and to treatment of disorders of cells, tissues and organisms. These aspects of the invention are discussed further below.

TRANSGENICS AND "KNOCK-OUTS"

[0118] The TR16 proteins of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

[0119] Any technique known in the art may be used to introduce the transgene (i.e., nucleic acids of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson *et al.*, *Appl. Microbiol. Biotechnol.* 40:691-698 (1994); Carver *et al.*, *Biotechnology (NY)* 11:1263-1270 (1993); Wright *et al.*, *Biotechnology (NY)* 9:830-834 (1991); and Hoppe *et al.*, US Patent Number 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten *et al.*, *Proc. Natl. Acad. Sci., USA* 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson *et al.*,

Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, *Mol Cell. Biol.* 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer *et al.*, *Science* 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano *et al.*, *Cell* 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," *Intl. Rev. Cytol.* 115:171-229 (1989), which is incorporated by reference herein in its entirety. Further, the contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety. Gordon, "Transgenic Animals," *Intl. Rev. Cytol.* 115:171-229 (1989), which is incorporated by reference herein in its entirety. See also, U.S. Patent No. 5,464,764 (Capecchi, et al., Positive-Negative Selection Methods and Vectors); U.S. Patent No. 5,631,153 (Capecchi, et al., Cells and Non-Human Organisms Containing Predetermined Genomic Modifications and Positive-Negative Selection Methods and Vectors for Making Same); U.S. Patent No. 4,736,866 (Leder, et al., Transgenic Non-Human Animals); and U.S. Patent No. 4,873,191 (Wagner, et al., Genetic Transformation of Zygotes); each of which is hereby incorporated by reference in its entirety.

[0120] Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campbell *et al.*, *Nature* 380:64-66 (1996); Wilmut *et al.*, *Nature* 385:810-813 (1997)), each of which is herein incorporated by reference in its entirety).

[0121] The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals or chimeric animals. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko *et al.* (*Proc. Natl. Acad. Sci. USA* 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting

is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu *et al.* (*Science* 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. The contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety.

[0122] Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

[0123] Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

[0124] Transgenic and "knock-out" animals of the invention have uses which include,

but are not limited to, animal model systems useful in elaborating the biological function of TR16 polypeptides, studying conditions and/or disorders associated with aberrant TR16-short and/or TR16-long expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

[0125] In further embodiments of the invention, cells that are genetically engineered to express the proteins of the invention, or alternatively, that are genetically engineered not to express the proteins of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells, etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally. Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. US Patent Number 5,399,349; and Mulligan & Wilson, US Patent Number 5,460,959, each of which is incorporated by reference herein in its entirety).

[0126] When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced

cells to be recognized by the host immune system.

TR16 RECEPTOR POLYPEPTIDES AND FRAGMENTS

[0127] The TR16 proteins (polypeptides) of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers, and higher multimers). Accordingly, the present invention relates to monomers and multimers of the TR16 proteins (polypeptides) of the invention, their preparation, and compositions (preferably, pharmaceutical compositions) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

[0128] Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only TR16 proteins of the invention (including TR16 fragments, variants, and fusion proteins, as described herein). These homomers may contain TR16 proteins having identical or different polypeptide sequences. In a specific embodiment, a homomer of the invention is a multimer containing only TR16 proteins having an identical polypeptide sequence. In another specific embodiment, a homomer of the invention is a multimer containing TR16 proteins having different polypeptide sequences (e.g., multimers containing proteins having both TR16-short and TR16-long polypeptide sequences). In specific embodiments, the multimer of the invention is a homodimer (e.g., containing TR16 proteins having identical or different polypeptide sequences) or a homotrimer (e.g., containing TR16 proteins having identical or different polypeptide sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

[0129] As used herein, the term heteromer refers to a multimer containing heterologous proteins (i.e., proteins containing only polypeptide sequences that do not correspond to a polypeptide sequences encoded by the TR16 gene) in addition to the TR16 proteins of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

[0130] Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic

and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when proteins of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when proteins of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the TR16 proteins of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence of the protein (e.g., the polypeptide sequence shown in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4), or a polypeptide encoded by one of the deposited cDNA clones). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a TR16 fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a TR16-Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequences from another TNF family ligand/receptor member that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more TR16 polypeptides of the invention are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple TR16 polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology.

[0131] Another method for preparing multimer TR16 polypeptides of the invention

involves use of TR16 polypeptides fused to a leucine zipper or isoleucine polypeptide sequence. Leucine zipper domains and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric TR16 proteins are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a soluble TR16 polypeptide fused to a peptide that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric TR16 is recovered from the culture supernatant using techniques known in the art.

[0132] Certain members of the TNF family of proteins are believed to exist in trimeric form (Beutler and Hufel, *Science* 264:667, 1994; Banner et al., *Cell* 73:431, 1993). Thus, trimeric TR16 may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (*FEBS Letters* 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922 (U.S. Patent No. 5,716,805), hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric TR16.

[0133] In further preferred embodiments, TR16 polynucleotides of the invention are fused to a polynucleotide encoding a "FLAG" polypeptide. Thus, an TR16-FLAG or an TR16-FLAG fusion protein is encompassed by the present invention. The FLAG antigenic polypeptide may be fused to an TR16 or an TR16 polypeptide of the invention at either or both the amino or the carboxy terminus. In preferred embodiments, an TR16-FLAG or an TR16-FLAG fusion protein is expressed from a pFLAG-CMV-5a or a pFLAG-CMV-1 expression vector (available from Sigma, St. Louis, MO, USA). See, Andersson, S., et al., *J. Biol. Chem.* 264:8222-29 (1989); Thomsen, D. R., et al., *Proc. Natl. Acad. Sci. USA*, 81:659-63 (1984); and Kozak, M., *Nature* 308:241 (1984) (each of which is hereby incorporated by reference). In further preferred embodiments, an TR16-FLAG or an TR16-FLAG fusion protein is detectable by anti-FLAG monoclonal antibodies (also available from Sigma). In a further embodiment, associated proteins of

the invention are associated by interactions between heterologous polypeptide sequence contained in FLAG-TR16 fusion proteins of the invention and anti-FLAG antibody.

[0134] The multimers of the invention may be generated using chemical techniques known in the art. For example, proteins desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the polypeptide sequence of the proteins desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, proteins of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide sequence of the protein and techniques known in the art may be applied to generate multimers containing one or more of these modified proteins (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the protein components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

[0135] Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, proteins contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain and

which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

[0136] The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the TR16 polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988).

[0137] Accordingly, in one embodiment, the invention provides an isolated TR16 polypeptide comprising, or alternatively consisting of, the amino acid sequence encoded by one or more of the deposited cDNAs, or the amino acid sequence in Figures 1A-E (SEQ ID NO:2), or the amino acid sequence in Figures 4A-E (SEQ ID NO:4), or a polypeptide comprising, or alternatively consisting of, a portion of the above polypeptides, such as for example, mature TR16-short (amino acids 48 to 963 of Figures 1A-E (SEQ ID NO:2)), mature TR16-long (amino acids 48 to 1027 of Figures 4A-E (SEQ ID NO:4)), the TR16 extracellular domain (amino acids 48 to 923 of Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4)), the TR16 cysteine rich domain (comprising amino acids 289 to 920 of Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4)), the TR16-short intracellular domain (amino acids 949 to 963 of Figures 1A-E), and/or the TR16-long intracellular domain (amino acids 949-1027 of Figures 4A-E (SEQ ID NO:4)).

[0138] Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 47, 48 to 80, 81 to 120, 121 to 160, 161 to 200, 201 to 240, 241 to 289, 290 to 320, 321 to 344, 345 to 355, 356 to 380, 381 to 426, 427 to 470, 471 to 500, 501 to 540, 541 to 580, 581 to 601, 602 to 640, 641 to 672, 673 to 710, 711 to 740, 741 to 780, 781 to 824, 825 to 870, 871 to 919, 920 to 923, 924 to 948, and/or 949 to 963 of SEQ ID NO:2 or SEQ ID NO:4. Additional representative examples of polypeptide fragments

of the invention, include, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 949 to 980, 981 to 1000, and/or 1001 to 1021 of SEQ ID NO:4. Moreover, polypeptide fragments can be at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 175, 200, 250, 300, 350, 400 or 500 amino acids in length. Polynucleotides encoding these polypeptides are also encompassed by the invention. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0139] In additional embodiments, the polypeptide fragments of the invention comprise, or alternatively consist of, one or more TR16 domains. Preferred polypeptide fragments of the present invention include a member selected from the group: (a) a polypeptide comprising or alternatively, consisting of, the TR16 extracellular domain (predicted to constitute amino acid residues from about 48 to about 923 Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4)); (b) a polypeptide comprising or alternatively, consisting of, a TR16 cysteine rich domain (predicted to constitute amino acid residues from about 289 to about 920 Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4)); (c) a polypeptide comprising or alternatively, consisting of, the TR16 transmembrane domain (predicted to constitute amino acid residues from about 924 to about 948 Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4)); (d) a polypeptide comprising or alternatively, consisting of, the TR16-short intracellular domain (predicted to constitute amino acid residues from about 949 to about 963 Figures 1A-E (SEQ ID NO:2)); (e) a polypeptide comprising or alternatively, consisting of, the TR16-long intracellular domain (predicted to constitute amino acid residues from about 949 to about 1027 Figures 4A-E (SEQ ID NO:4)); (f) a polypeptide comprising, or alternatively, consisting of, one, two, three, four or more, epitope bearing portions of the TR16-short protein; or (g) any combination of polypeptides (a)-(f). Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0140] As discussed above, it is believed that the extracellular cysteine rich motifs of TR16 are important for interactions between TR16 and its ligands. Accordingly, in preferred embodiments, polypeptide fragments of the invention comprise, or alternatively consist of amino acid residues 290 to 344, 356 to 426, 602 to 672, and/or 825 to 919 of Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4). In a specific embodiment

the polypeptides of the invention comprise, or alternatively consist of any combination of one, two, three or all four of the extracellular cysteine rich motifs disclosed in Figures 1A-E or Figures 4A-E. Proteins comprising or alternatively consisting of a polypeptide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide sequences of one, two, three, or all four of these cysteine rich motifs are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0141] Among the especially preferred fragments of the invention are fragments characterized by structural or functional attributes of TR16. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) TR16 (Figures 1A-E (SEQ ID NO:2) and Figures 4A-E (SEQ ID NO:4)). Certain preferred regions are those set out in Figures 3 and 5 and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence depicted in Figures 1A-E (SEQ ID NO:2) and Figures 4A-E (SEQ ID NO:4), respectively, such preferred regions include; Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle predicted hydrophilic; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0142] Polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of: an amino acid sequence contained in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); an amino acid sequence encoded by a cDNA contained in ATCC Deposit No. PTA-506; an amino acid or encoded by a nucleic acid containing a polynucleotide sequence which hybridizes (e.g., under stringent hybridization conditions) to the cDNA sequence contained in a deposited clone; an amino

acid sequence encoded by a nucleic acid containing a polynucleotide sequence which hybridizes to the complementary strand of the nucleotide sequence shown in Figures 1A-E (SEQ ID NO:1) or Figures 4A-E (SEQ ID NO:3; and an amino acid sequence encoded by a nucleic acid containing a polynucleotide sequence which hybridizes to the complementary strand of a polynucleotide sequence encoding a polypeptide selected from the group consisting of: PCQEKDYH (SEQ ID NO:7), GKECTFSC (SEQ ID NO:8), GCNNSSWI (SEQ ID NO:9), FEFFIQND (SEQ ID NO:10), GSHSVMLK (SEQ ID NO:11), TIEGVAYT (SEQ ID NO:12), SQFSGSSE (SEQ ID NO:13), EEGKTQIM (SEQ ID NO:14), DGTKECRP (SEQ ID NO:15), DGMNGWEV (SEQ ID NO:16), PGFKPPTS (SEQ ID NO:17), YFMVDINR (SEQ ID NO:18), QCQDNRRF (SEQ ID NO:19), KNNQDHSV (SEQ ID NO:20), CGHEGKKM (SEQ ID NO:21), DTFIGVTV (SEQ ID NO:22), FFYKSSTA (SEQ ID NO:23), ISVPSKCP (SEQ ID NO:24), and/or RGFQETLY (SEQ ID NO:25), KNQKKKKT (SEQ ID NO:26), KNQKLEYK (SEQ ID NO:27), and LATKEKED (SEQ ID NO:28) of Figures 4A-E (SEQ ID NO:4). Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0143] In another specific embodiment, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence encoded by a nucleic acid containing a polynucleotide sequence which hybridizes (e.g., under stringent hybridization conditions) to the complementary strand of a polynucleotide sequence encoding a polypeptide selected from the group consisting of: MAPWNVLPGPFPFHSSRLHGSGHSRLAAAAISIALKAFSCASG (SEQ ID NO:29), TIEEGSSE (SEQ ID NO:30), CTERPPCTTKDYFQIHTPCDEEGKTQIMYKWIEP KICREDLTDAIRLPPSGEKKDCPPCNPGFYNNGSSSCHPC (SEQ ID NO:31), TKGW WIISGSSSLRRTFKHAF CSTFAAEC (SEQ ID NO:32), FKMDGIIYSKRFKHITIVMW TQCLQRVWTGMIKPP (SEQ ID NO:33), and QDNRPIPPLSISIVPYVSIVAGLILWI SIDVTFPRRF (SEQ ID NO:34). Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0144] In another specific embodiment, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence encoded by a nucleic acid containing a polynucleotide sequence which hybridizes (e.g., under stringent hybridization conditions) to the complementary strand of a nucleotide sequence encoding the amino acid sequence: KNQKLEYKYSKLVMTTNSK

ECELPAADSCAIMEGEDNEEEVVYSNKQSLLGKLKSLATKEKEDHFESVQLKTSR SPNI (SEQ ID NO:35). Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0145] In additional specific embodiments, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence selected from the group consisting of: PCQEKDYH (SEQ ID NO:7), GKECTFSC (SEQ ID NO:8), GCNNSSWI (SEQ ID NO:9), FEFFIQND (SEQ ID NO:10), GSHSVMLK (SEQ ID NO:11), TIEGVAYT (SEQ ID NO:12), SQFSGSSE (SEQ ID NO:13), EEGKTQIM (SEQ ID NO:14), DGTKECRP (SEQ ID NO:15), DGMNGWEV (SEQ ID NO:16), PGFKPPTS (SEQ ID NO:17), YFMVDINR (SEQ ID NO:18), QCQDNRRF (SEQ ID NO:19), KNNQDHSV (SEQ ID NO:20), CGHEGKKM (SEQ ID NO:21), DTFIGVTV (SEQ ID NO:22), FFYKSSTA (SEQ ID NO:23), ISVPSKCP (SEQ ID NO:24), RGFQETLY (SEQ ID NO:25) of SEQ ID NO:2 or Figures 4A-E (SEQ ID NO:4); KNQKKKKT (SEQ ID NO:26) of SEQ ID NO:2; KNQKLEYK (SEQ ID NO:27), LATKEKED (SEQ ID NO:28). Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0146] In a specific embodiment, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, a polypeptide sequence selected from the group consisting of: MAPWNVLPGPFPHPHSSRLHGSGHSRLAAAA ISIALK AFSCASG (SEQ ID NO:29), TIEEGSSE (SEQ ID NO:30), CTERPPCTT KDYFQIHTPCDEEGKTQIMYKWIEPKICREDLTDAIRLPPSGEKKDCPPCNPGFYN GSSSCHPC (SEQ ID NO:31), TKGWWIISGSSSLRRTFKHAF CSTFAAEC (SEQ ID NO:32), FKMDGIIYSKRFKHITIVMWTQCLQRVWTGMIKPP (SEQ ID NO:33), and QDNRPPIPLSISIVPYVSIVAGLILWISIDVTFPRRF (SEQ ID NO:34). Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

[0147] In another specific embodiment, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, the amino acid sequence consisting of: KNQKLEYKYSKLVMTTNSKECELPAADSCAIMEGEDNEEE VVYSNKQSLLGKLKSLATKEKEDHFESVQLKTSRSPNI (SEQ ID NO:35). Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0148] As mentioned above, even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions

of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind TR16 ligand (e.g., Neutrokin- α)) may still be retained. For example, the ability of shortened TR16 muteins to induce and/or bind to antibodies which recognize the complete (full-length) or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete full-length polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that an TR16 mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six TR16 amino acid residues may often evoke an immune response.

[0149] Accordingly, the present invention provides polypeptides having one or more residues deleted from the amino terminus of the TR16-short amino acid sequence shown in Figures 1A-E, up to the isoleucine residue at position number 958 and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n^1 -963 of Figures 1A-E, where n^1 is an integer from 2 to 958 corresponding to the position of the amino acid residue in Figures 1A-E (SEQ ID NO:2).

[0150] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues: L-2 to N-963; F-3 to N-963; R-4 to N-963; A-5 to N-963; R-6 to N-963; G-7 to N-963; P-8 to N-963; V-9 to N-963; R-10 to N-963; G-11 to N-963; R-12 to N-963; G-13 to N-963; W-14 to N-963; G-15 to N-963; R-16 to N-963; P-17 to N-963; A-18 to N-963; E-19 to N-963; A-20 to N-963; P-21 to N-963; R-22 to N-963; R-23 to N-963; G-24 to N-963; R-25 to N-963; S-26 to N-963; P-27 to N-963; P-28 to N-963; W-29 to N-963; S-30 to N-963; P-31 to N-963; A-32 to N-963; W-33 to N-963; I-34 to N-963; C-35 to N-963; C-36 to N-963; W-37 to N-963; A-38 to N-963; L-39 to N-963; A-40 to N-963; G-41 to N-963; C-42 to N-963; Q-43 to N-963; A-44 to N-963; A-45 to N-963; W-46 to N-963; A-47 to N-963; G-48 to N-963; D-49 to N-963; L-50 to N-963; P-51 to N-963; S-52 to N-963; S-53 to N-963; S-54 to N-963; S-55 to N-963; R-56 to N-963; P-57 to N-963; L-58 to N-963; P-59 to N-963; P-60 to N-963; C-61 to N-963; Q-62 to N-963; E-63 to N-963; K-64

to N-963; D-65 to N-963; Y-66 to N-963; H-67 to N-963; F-68 to N-963; E-69 to N-963; Y-70 to N-963; T-71 to N-963; E-72 to N-963; C-73 to N-963; D-74 to N-963; S-75 to N-963; S-76 to N-963; G-77 to N-963; S-78 to N-963; R-79 to N-963; W-80 to N-963; R-81 to N-963; V-82 to N-963; A-83 to N-963; I-84 to N-963; P-85 to N-963; N-86 to N-963; S-87 to N-963; A-88 to N-963; V-89 to N-963; D-90 to N-963; C-91 to N-963; S-92 to N-963; G-93 to N-963; L-94 to N-963; P-95 to N-963; D-96 to N-963; P-97 to N-963; V-98 to N-963; R-99 to N-963; G-100 to N-963; K-101 to N-963; E-102 to N-963; C-103 to N-963; T-104 to N-963; F-105 to N-963; S-106 to N-963; C-107 to N-963; A-108 to N-963; S-109 to N-963; G-110 to N-963; E-111 to N-963; Y-112 to N-963; L-113 to N-963; E-114 to N-963; M-115 to N-963; K-116 to N-963; N-117 to N-963; Q-118 to N-963; V-119 to N-963; C-120 to N-963; S-121 to N-963; K-122 to N-963; C-123 to N-963; G-124 to N-963; E-125 to N-963; G-126 to N-963; T-127 to N-963; Y-128 to N-963; S-129 to N-963; L-130 to N-963; G-131 to N-963; S-132 to N-963; G-133 to N-963; I-134 to N-963; K-135 to N-963; F-136 to N-963; D-137 to N-963; E-138 to N-963; W-139 to N-963; D-140 to N-963; E-141 to N-963; L-142 to N-963; P-143 to N-963; A-144 to N-963; G-145 to N-963; F-146 to N-963; S-147 to N-963; N-148 to N-963; I-149 to N-963; A-150 to N-963; T-151 to N-963; F-152 to N-963; M-153 to N-963; D-154 to N-963; T-155 to N-963; V-156 to N-963; V-157 to N-963; G-158 to N-963; P-159 to N-963; S-160 to N-963; D-161 to N-963; S-162 to N-963; R-163 to N-963; P-164 to N-963; D-165 to N-963; G-166 to N-963; C-167 to N-963; N-168 to N-963; N-169 to N-963; S-170 to N-963; S-171 to N-963; W-172 to N-963; I-173 to N-963; P-174 to N-963; R-175 to N-963; G-176 to N-963; N-177 to N-963; Y-178 to N-963; I-179 to N-963; E-180 to N-963; S-181 to N-963; N-182 to N-963; R-183 to N-963; D-184 to N-963; D-185 to N-963; C-186 to N-963; T-187 to N-963; V-188 to N-963; S-189 to N-963; L-190 to N-963; I-191 to N-963; Y-192 to N-963; A-193 to N-963; V-194 to N-963; H-195 to N-963; L-196 to N-963; K-197 to N-963; K-198 to N-963; S-199 to N-963; G-200 to N-963; Y-201 to N-963; V-202 to N-963; F-203 to N-963; F-204 to N-963; E-205 to N-963; Y-206 to N-963; Q-207 to N-963; Y-208 to N-963; V-209 to N-963; D-210 to N-963; N-211 to N-963; N-212 to N-963; I-213 to N-963; F-214 to N-963; F-215 to N-963; E-216 to N-963; F-217 to N-963; F-218 to N-963; I-219 to N-963; Q-220 to N-963; N-221 to N-963; D-222 to N-963; Q-223 to N-963; C-224 to N-963; Q-225 to N-963; E-226 to N-963; M-227 to N-963; D-228 to N-963; T-229 to N-963; T-230 to N-963; T-231 to N-963; D-232 to N-963; K-233 to N-963; W-234 to

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[0153] In another embodiment, N-terminal deletions of the TR16 polypeptide can be described by the general formula n^2 -923, where n^2 is a number from 2 to 919, corresponding to the position of amino acid identified in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4). Preferably, N-terminal deletions of the TR16-short or TR16-long polypeptide of the invention shown as Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4) respectively include polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues: L-2 to D-923; F-3 to D-923; R-4 to D-923; A-5 to D-923; R-6 to D-923; G-7 to D-923; P-8 to D-923; V-9 to D-923; R-10 to D-923; G-11 to D-923; R-12 to D-923; G-13 to D-923; W-14 to D-923; G-15 to D-923; R-16 to D-923; P-17 to D-923; A-18 to D-923; E-19 to D-923; A-20 to D-923; P-21 to D-923; R-22 to D-923; R-23 to D-923; G-24 to D-923; R-25 to D-923; S-26 to D-923; P-27 to D-923; P-28 to D-923; W-29 to D-923; S-30 to D-923; P-31 to D-923; A-32 to D-923; W-33 to D-923; I-34 to D-923; C-35 to D-923; C-36 to D-923; W-37 to D-923; A-38 to D-923; L-39 to D-923; A-40 to D-923; G-41 to D-923; C-42 to D-923; Q-43 to D-923; A-44 to D-923; A-45 to D-923; W-46 to D-923; A-47 to D-923; G-48 to D-923; D-49 to D-923; L-50 to D-923; P-51 to D-923; S-52 to D-923; S-53 to D-923; S-54 to D-923; S-55 to D-923; R-56 to D-923; P-57 to D-923; L-58 to D-923; P-59 to D-923; P-60 to D-923; C-61 to D-923; Q-62 to D-923; E-63 to D-923; K-64 to D-923; D-65 to D-923; Y-66 to D-923; H-67 to D-923; F-68 to D-923; E-69 to D-923; Y-70 to D-923; T-71 to D-923; E-72 to D-923; C-73 to D-923; D-74 to D-923; S-75 to D-923; S-76 to D-923; G-77 to D-923; S-78 to D-923; R-79 to D-923; W-80 to D-923; R-81 to D-923; V-82 to D-923;

A-83 to D-923; I-84 to D-923; P-85 to D-923; N-86 to D-923; S-87 to D-923; A-88 to D-923; V-89 to D-923; D-90 to D-923; C-91 to D-923; S-92 to D-923; G-93 to D-923; L-94 to D-923; P-95 to D-923; D-96 to D-923; P-97 to D-923; V-98 to D-923; R-99 to D-923; G-100 to D-923; K-101 to D-923; E-102 to D-923; C-103 to D-923; T-104 to D-923; F-105 to D-923; S-106 to D-923; C-107 to D-923; A-108 to D-923; S-109 to D-923; G-110 to D-923; E-111 to D-923; Y-112 to D-923; L-113 to D-923; E-114 to D-923; M-115 to D-923; K-116 to D-923; N-117 to D-923; Q-118 to D-923; V-119 to D-923; C-120 to D-923; S-121 to D-923; K-122 to D-923; C-123 to D-923; G-124 to D-923; E-125 to D-923; G-126 to D-923; T-127 to D-923; Y-128 to D-923; S-129 to D-923; L-130 to D-923; G-131 to D-923; S-132 to D-923; G-133 to D-923; I-134 to D-923; K-135 to D-923; F-136 to D-923; D-137 to D-923; E-138 to D-923; W-139 to D-923; D-140 to D-923; E-141 to D-923; L-142 to D-923; P-143 to D-923; A-144 to D-923; G-145 to D-923; F-146 to D-923; S-147 to D-923; N-148 to D-923; I-149 to D-923; A-150 to D-923; T-151 to D-923; F-152 to D-923; M-153 to D-923; D-154 to D-923; T-155 to D-923; V-156 to D-923; V-157 to D-923; G-158 to D-923; P-159 to D-923; S-160 to D-923; D-161 to D-923; S-162 to D-923; R-163 to D-923; P-164 to D-923; D-165 to D-923; G-166 to D-923; C-167 to D-923; N-168 to D-923; N-169 to D-923; S-170 to D-923; S-171 to D-923; W-172 to D-923; I-173 to D-923; P-174 to D-923; R-175 to D-923; G-176 to D-923; N-177 to D-923; Y-178 to D-923; I-179 to D-923; E-180 to D-923; S-181 to D-923; N-182 to D-923; R-183 to D-923; D-184 to D-923; D-185 to D-923; C-186 to D-923; T-187 to D-923; V-188 to D-923; S-189 to D-923; L-190 to D-923; I-191 to D-923; Y-192 to D-923; A-193 to D-923; V-194 to D-923; H-195 to D-923; L-196 to D-923; K-197 to D-923; K-198 to D-923; S-199 to D-923; G-200 to D-923; Y-201 to D-923; V-202 to D-923; F-203 to D-923; F-204 to D-923; E-205 to D-923; Y-206 to D-923; Q-207 to D-923; Y-208 to D-923; V-209 to D-923; D-210 to D-923; N-211 to D-923; N-212 to D-923; I-213 to D-923; F-214 to D-923; F-215 to D-923; E-216 to D-923; F-217 to D-923; F-218 to D-923; I-219 to D-923; Q-220 to D-923; N-221 to D-923; D-222 to D-923; Q-223 to D-923; C-224 to D-923; Q-225 to D-923; E-226 to D-923; M-227 to D-923; D-228 to D-923; T-229 to D-923; T-230 to D-923; T-231 to D-923; D-232 to D-923; K-233 to D-923; W-234 to D-923; V-235 to D-923; K-236 to D-923; L-237 to D-923; T-238 to D-923; D-239 to D-923; N-240 to D-923; G-241 to D-923; E-242 to D-923; W-243 to D-923; G-244 to D-923; S-245 to D-923; H-246 to D-923; S-247 to D-923; V-248 to D-923; M-249 to D-923; L-250 to D-923; K-

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or TR16-long extracellular domain sequence shown in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4) respectively. The present invention is also directed to nucleic acid molecules comprising or, alternatively, consisting of a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequences encoding the TR16 polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0154] Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind TR16 ligand (e.g., Neutrokin- α) may still be retained). For example the ability of a TR16 mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a TR16 mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six TR16 amino acid residues may often evoke an immune response.

[0155] Accordingly, the present invention provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the TR16-short polypeptide shown in Figures 1A-E, up to the arginine residue at position number 6, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues 1-m¹ of Figures 1A-E, where m¹ is an integer from 6 to 962 corresponding to the position of the amino acid residue in Figures 1A-E (SEQ ID NO:2).

[0156] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues: M-1 to F-962; M-1 to L-961; M-1 to N-960; M-1 to L-959; M-1 to I-958; M-1 to T-957; M-1 to K-956; M-1 to K-955; M-1 to K-954; M-1 to K-953; M-1 to Q-952; M-1 to

N-951; M-1 to K-950; M-1 to K-949; M-1 to W-948; M-1 to F-947; M-1 to Y-946; M-1 to C-945; M-1 to T-944; M-1 to L-943; M-1 to A-942; M-1 to V-941; M-1 to L-940; M-1 to L-939; M-1 to V-938; M-1 to A-937; M-1 to T-936; M-1 to F-935; M-1 to A-934; M-1 to G-933; M-1 to V-932; M-1 to G-931; M-1 to A-930; M-1 to G-929; M-1 to V-928; M-1 to K-927; M-1 to L-926; M-1 to W-925; M-1 to F-924; M-1 to D-923; M-1 to V-922; M-1 to T-921; M-1 to E-920; M-1 to C-919; M-1 to T-918; M-1 to A-917; M-1 to L-916; M-1 to K-915; M-1 to K-914; M-1 to E-913; M-1 to P-912; M-1 to L-911; M-1 to S-910; M-1 to I-909; M-1 to G-908; M-1 to K-907; M-1 to I-906; M-1 to C-905; M-1 to W-904; M-1 to K-903; M-1 to P-902; M-1 to E-901; M-1 to N-900; M-1 to W-899; M-1 to V-898; M-1 to Y-897; M-1 to L-896; M-1 to T-895; M-1 to E-894; M-1 to Q-893; M-1 to F-892; M-1 to G-891; M-1 to R-890; M-1 to K-889; M-1 to C-888; M-1 to A-887; M-1 to G-886; M-1 to E-885; M-1 to I-884; M-1 to E-883; M-1 to H-882; M-1 to F-881; M-1 to D-880; M-1 to H-879; M-1 to E-878; M-1 to T-877; M-1 to C-876; M-1 to L-875; M-1 to P-874; M-1 to C-873; M-1 to A-872; M-1 to E-871; M-1 to A-870; M-1 to S-869; M-1 to E-868; M-1 to W-867; M-1 to L-866; M-1 to F-865; M-1 to Y-864; M-1 to F-863; M-1 to T-862; M-1 to C-861; M-1 to G-860; M-1 to D-859; M-1 to C-858; M-1 to T-857; M-1 to G-856; M-1 to A-855; M-1 to P-854; M-1 to C-853; M-1 to K-852; M-1 to S-851; M-1 to P-850; M-1 to V-849; M-1 to S-848; M-1 to I-847; M-1 to V-846; M-1 to G-845; M-1 to A-844; M-1 to G-843; M-1 to S-842; M-1 to K-841; M-1 to T-840; M-1 to P-839; M-1 to N-838; M-1 to C-837; M-1 to R-836; M-1 to M-835; M-1 to K-834; M-1 to V-833; M-1 to A-832; M-1 to T-831; M-1 to S-830; M-1 to R-829; M-1 to G-828; M-1 to N-827; M-1 to I-826; M-1 to C-825; M-1 to S-824; M-1 to T-823; M-1 to T-822; M-1 to A-821; M-1 to T-820; M-1 to S-819; M-1 to S-818; M-1 to K-817; M-1 to Y-816; M-1 to F-815; M-1 to F-814; M-1 to H-813; M-1 to V-812; M-1 to D-811; M-1 to P-810; M-1 to I-809; M-1 to Q-808; M-1 to S-807; M-1 to T-806; M-1 to P-805; M-1 to V-804; M-1 to P-803; M-1 to F-802; M-1 to M-801; M-1 to D-800; M-1 to E-799; M-1 to K-798; M-1 to I-797; M-1 to N-796; M-1 to I-795; M-1 to N-794; M-1 to K-793; M-1 to L-792; M-1 to T-791; M-1 to T-790; M-1 to E-789; M-1 to V-788; M-1 to T-787; M-1 to V-786; M-1 to G-785; M-1 to I-784; M-1 to F-783; M-1 to T-782; M-1 to D-781; M-1 to A-780; M-1 to L-779; M-1 to I-778; M-1 to I-777; M-1 to S-776; M-1 to Q-775; M-1 to S-774; M-1 to S-773; M-1 to L-772; M-1 to A-771; M-1 to A-770; M-1 to R-769; M-1 to F-768; M-1 to G-767; M-1 to K-766; M-1 to S-765; M-1 to E-764; M-1 to S-763; M-1 to P-762; M-1 to I-761; M-1 to I-760; M-1 to T-

759; M-1 to S-758; M-1 to Q-757; M-1 to C-756; M-1 to V-755; M-1 to F-754; M-1 to A-753; M-1 to G-752; M-1 to V-751; M-1 to L-750; M-1 to N-749; M-1 to T-748; M-1 to Y-747; M-1 to D-746; M-1 to D-745; M-1 to S-744; M-1 to G-743; M-1 to A-742; M-1 to V-741; M-1 to I-740; M-1 to E-739; M-1 to K-738; M-1 to V-737; M-1 to T-736; M-1 to F-735; M-1 to D-734; M-1 to T-733; M-1 to I-732; M-1 to N-731; M-1 to N-730; M-1 to T-729; M-1 to C-728; M-1 to L-727; M-1 to A-726; M-1 to M-725; M-1 to K-724; M-1 to K-723; M-1 to G-722; M-1 to E-721; M-1 to H-720; M-1 to G-719; M-1 to C-718; M-1 to L-717; M-1 to S-716; M-1 to I-715; M-1 to N-714; M-1 to F-713; M-1 to F-712; M-1 to H-711; M-1 to F-710; M-1 to Y-709; M-1 to K-708; M-1 to T-707; M-1 to G-706; M-1 to K-705; M-1 to S-704; M-1 to T-703; M-1 to F-702; M-1 to S-701; M-1 to P-700; M-1 to G-699; M-1 to N-698; M-1 to M-697; M-1 to L-696; M-1 to S-695; M-1 to G-694; M-1 to V-693; M-1 to S-692; M-1 to S-691; M-1 to L-690; M-1 to N-689; M-1 to S-688; M-1 to F-687; M-1 to D-686; M-1 to Y-685; M-1 to H-684; M-1 to L-683; M-1 to I-682; M-1 to Q-681; M-1 to N-680; M-1 to E-679; M-1 to K-678; M-1 to E-677; M-1 to H-676; M-1 to Y-675; M-1 to F-674; M-1 to F-673; M-1 to C-672; M-1 to D-671; M-1 to S-670; M-1 to Y-669; M-1 to C-668; M-1 to V-667; M-1 to S-666; M-1 to H-665; M-1 to D-664; M-1 to Q-663; M-1 to N-662; M-1 to N-661; M-1 to K-660; M-1 to S-659; M-1 to G-658; M-1 to P-657; M-1 to G-656; M-1 to C-655; M-1 to P-654; M-1 to I-653; M-1 to C-652; M-1 to A-651; M-1 to E-650; M-1 to K-649; M-1 to G-648; M-1 to Y-647; M-1 to V-646; M-1 to Q-645; M-1 to H-644; M-1 to I-643; M-1 to S-642; M-1 to L-641; M-1 to Y-640; M-1 to T-639; M-1 to D-638; M-1 to P-637; M-1 to P-636; M-1 to C-635; M-1 to E-634; M-1 to K-633; M-1 to C-632; M-1 to Q-631; M-1 to N-630; M-1 to T-629; M-1 to E-628; M-1 to K-627; M-1 to E-626; M-1 to I-625; M-1 to Y-624; M-1 to H-623; M-1 to G-622; M-1 to P-621; M-1 to P-620; M-1 to C-619; M-1 to P-618; M-1 to V-617; M-1 to C-616; M-1 to S-615; M-1 to S-614; M-1 to G-613; M-1 to S-612; M-1 to Q-611; M-1 to E-610; M-1 to S-609; M-1 to G-608; M-1 to L-607; M-1 to A-606; M-1 to C-605; M-1 to A-604; M-1 to R-603; M-1 to C-602; M-1 to S-601; M-1 to S-600; M-1 to A-599; M-1 to V-598; M-1 to G-597; M-1 to D-596; M-1 to V-595; M-1 to A-594; M-1 to N-593; M-1 to T-592; M-1 to A-591; M-1 to T-590; M-1 to I-589; M-1 to S-588; M-1 to Y-587; M-1 to I-586; M-1 to K-585; M-1 to V-584; M-1 to M-583; M-1 to D-582; M-1 to N-581; M-1 to I-580; M-1 to F-579; M-1 to R-578; M-1 to R-577; M-1 to N-576; M-1 to D-575; M-1 to Q-574; M-1 to G-573; M-1 to Q-572; M-1 to N-571; M-1 to T-570; M-1 to R-569; M-1 to Q-568; M-1 to

F-567; M-1 to A-566; M-1 to W-565; M-1 to T-564; M-1 to F-563; M-1 to T-562; M-1 to F-561; M-1 to T-560; M-1 to A-559; M-1 to N-558; M-1 to K-557; M-1 to F-556; M-1 to I-555; M-1 to I-554; M-1 to H-553; M-1 to T-552; M-1 to Y-551; M-1 to A-550; M-1 to Q-549; M-1 to K-548; M-1 to E-547; M-1 to K-546; M-1 to T-545; M-1 to G-544; M-1 to G-543; M-1 to W-542; M-1 to S-541; M-1 to E-540; M-1 to V-539; M-1 to V-538; M-1 to N-537; M-1 to T-536; M-1 to S-535; M-1 to K-534; M-1 to R-533; M-1 to N-532; M-1 to I-531; M-1 to D-530; M-1 to V-529; M-1 to M-528; M-1 to F-527; M-1 to Y-526; M-1 to L-525; M-1 to V-524; M-1 to C-523; M-1 to D-522; M-1 to A-521; M-1 to S-520; M-1 to C-519; M-1 to L-518; M-1 to T-517; M-1 to E-516; M-1 to F-515; M-1 to V-514; M-1 to F-513; M-1 to T-512; M-1 to I-511; M-1 to R-510; M-1 to G-509; M-1 to L-508; M-1 to E-507; M-1 to S-506; M-1 to G-505; M-1 to T-504; M-1 to A-503; M-1 to G-502; M-1 to T-501; M-1 to M-500; M-1 to S-499; M-1 to T-498; M-1 to P-497; M-1 to P-496; M-1 to K-495; M-1 to F-494; M-1 to G-493; M-1 to P-492; M-1 to I-491; M-1 to H-490; M-1 to L-489; M-1 to N-488; M-1 to L-487; M-1 to I-486; M-1 to L-485; M-1 to Y-484; M-1 to D-483; M-1 to N-482; M-1 to D-481; M-1 to S-480; M-1 to G-479; M-1 to G-478; M-1 to A-477; M-1 to G-476; M-1 to S-475; M-1 to Q-474; M-1 to I-473; M-1 to H-472; M-1 to D-471; M-1 to G-470; M-1 to A-469; M-1 to V-468; M-1 to E-467; M-1 to W-466; M-1 to G-465; M-1 to N-464; M-1 to M-463; M-1 to G-462; M-1 to D-461; M-1 to C-460; M-1 to K-459; M-1 to S-458; M-1 to N-457; M-1 to G-456; M-1 to V-455; M-1 to N-454; M-1 to F-453; M-1 to C-452; M-1 to S-451; M-1 to T-450; M-1 to K-449; M-1 to M-448; M-1 to N-447; M-1 to G-446; M-1 to P-445; M-1 to L-444; M-1 to V-443; M-1 to N-442; M-1 to W-441; M-1 to W-440; M-1 to K-439; M-1 to Y-438; M-1 to E-437; M-1 to F-436; M-1 to G-435; M-1 to L-434; M-1 to A-433; M-1 to P-432; M-1 to E-431; M-1 to T-430; M-1 to G-429; M-1 to A-428; M-1 to P-427; M-1 to C-426; M-1 to P-425; M-1 to R-424; M-1 to C-423; M-1 to E-422; M-1 to K-421; M-1 to T-420; M-1 to G-419; M-1 to D-418; M-1 to S-417; M-1 to F-416; M-1 to T-415; M-1 to G-414; M-1 to P-413; M-1 to P-412; M-1 to C-411; M-1 to P-410; M-1 to H-409; M-1 to C-408; M-1 to S-407; M-1 to S-406; M-1 to S-405; M-1 to G-404; M-1 to N-403; M-1 to N-402; M-1 to Y-401; M-1 to F-400; M-1 to G-399; M-1 to P-398; M-1 to N-397; M-1 to C-396; M-1 to P-395; M-1 to P-394; M-1 to C-393; M-1 to D-392; M-1 to K-391; M-1 to K-390; M-1 to E-389; M-1 to G-388; M-1 to S-387; M-1 to P-386; M-1 to P-385; M-1 to L-384; M-1 to R-383; M-1 to I-382; M-1 to A-381; M-1 to D-380; M-1 to T-379; M-1 to L-378; M-1 to D-377; M-1 to E-376;

M-1 to R-375; M-1 to C-374; M-1 to I-373; M-1 to K-372; M-1 to P-371; M-1 to E-370; M-1 to I-369; M-1 to W-368; M-1 to K-367; M-1 to Y-366; M-1 to M-365; M-1 to I-364; M-1 to Q-363; M-1 to T-362; M-1 to K-361; M-1 to G-360; M-1 to E-359; M-1 to E-358; M-1 to D-357; M-1 to C-356; M-1 to P-355; M-1 to T-354; M-1 to H-353; M-1 to I-352; M-1 to Q-351; M-1 to F-350; M-1 to Y-349; M-1 to D-348; M-1 to K-347; M-1 to T-346; M-1 to T-345; M-1 to C-344; M-1 to P-343; M-1 to P-342; M-1 to R-341; M-1 to E-340; M-1 to T-339; M-1 to C-338; M-1 to E-337; M-1 to S-336; M-1 to S-335; M-1 to G-334; M-1 to S-333; M-1 to F-332; M-1 to Q-331; M-1 to S-330; M-1 to D-329; M-1 to D-328; M-1 to K-327; M-1 to C-326; M-1 to R-325; M-1 to I-324; M-1 to C-323; M-1 to E-322; M-1 to K-321; M-1 to A-320; M-1 to G-319; M-1 to K-318; M-1 to E-317; M-1 to S-316; M-1 to Y-315; M-1 to T-314; M-1 to N-313; M-1 to R-312; M-1 to P-311; M-1 to C-310; M-1 to V-309; M-1 to Q-308; M-1 to C-307; M-1 to N-306; M-1 to F-305; M-1 to S-304; M-1 to G-303; M-1 to P-302; M-1 to K-301; M-1 to N-300; M-1 to S-299; M-1 to F-298; M-1 to T-297; M-1 to G-296; M-1 to P-295; M-1 to K-294; M-1 to C-293; M-1 to P-292; M-1 to F-291; M-1 to C-290; M-1 to E-289; M-1 to S-288; M-1 to T-287; M-1 to Y-286; M-1 to A-285; M-1 to V-284; M-1 to G-283; M-1 to E-282; M-1 to I-281; M-1 to T-280; M-1 to I-279; M-1 to N-278; M-1 to K-277; M-1 to V-276; M-1 to L-275; M-1 to V-274; M-1 to P-273; M-1 to K-272; M-1 to V-271; M-1 to A-270; M-1 to K-269; M-1 to S-268; M-1 to G-267; M-1 to M-266; M-1 to L-265; M-1 to I-264; M-1 to G-263; M-1 to T-262; M-1 to T-261; M-1 to R-260; M-1 to W-259; M-1 to Y-258; M-1 to L-257; M-1 to I-256; M-1 to N-255; M-1 to T-254; M-1 to G-253; M-1 to S-252; M-1 to K-251; M-1 to L-250; M-1 to M-249; M-1 to V-248; M-1 to S-247; M-1 to H-246; M-1 to S-245; M-1 to G-244; M-1 to W-243; M-1 to E-242; M-1 to G-241; M-1 to N-240; M-1 to D-239; M-1 to T-238; M-1 to L-237; M-1 to K-236; M-1 to V-235; M-1 to W-234; M-1 to K-233; M-1 to D-232; M-1 to T-231; M-1 to T-230; M-1 to T-229; M-1 to D-228; M-1 to M-227; M-1 to E-226; M-1 to Q-225; M-1 to C-224; M-1 to Q-223; M-1 to D-222; M-1 to N-221; M-1 to Q-220; M-1 to I-219; M-1 to F-218; M-1 to F-217; M-1 to E-216; M-1 to F-215; M-1 to F-214; M-1 to I-213; M-1 to N-212; M-1 to N-211; M-1 to D-210; M-1 to V-209; M-1 to Y-208; M-1 to Q-207; M-1 to Y-206; M-1 to E-205; M-1 to F-204; M-1 to F-203; M-1 to V-202; M-1 to Y-201; M-1 to G-200; M-1 to S-199; M-1 to K-198; M-1 to K-197; M-1 to L-196; M-1 to H-195; M-1 to V-194; M-1 to A-193; M-1 to Y-192; M-1 to I-191; M-1 to L-190; M-1 to S-189; M-1 to V-188; M-1 to T-187; M-1 to C-186; M-1 to D-185; M-1 to

D-184; M-1 to R-183; M-1 to N-182; M-1 to S-181; M-1 to E-180; M-1 to I-179; M-1 to Y-178; M-1 to N-177; M-1 to G-176; M-1 to R-175; M-1 to P-174; M-1 to I-173; M-1 to W-172; M-1 to S-171; M-1 to S-170; M-1 to N-169; M-1 to N-168; M-1 to C-167; M-1 to G-166; M-1 to D-165; M-1 to P-164; M-1 to R-163; M-1 to S-162; M-1 to D-161; M-1 to S-160; M-1 to P-159; M-1 to G-158; M-1 to V-157; M-1 to V-156; M-1 to T-155; M-1 to D-154; M-1 to M-153; M-1 to F-152; M-1 to T-151; M-1 to A-150; M-1 to I-149; M-1 to N-148; M-1 to S-147; M-1 to F-146; M-1 to G-145; M-1 to A-144; M-1 to P-143; M-1 to L-142; M-1 to E-141; M-1 to D-140; M-1 to W-139; M-1 to E-138; M-1 to D-137; M-1 to F-136; M-1 to K-135; M-1 to I-134; M-1 to G-133; M-1 to S-132; M-1 to G-131; M-1 to L-130; M-1 to S-129; M-1 to Y-128; M-1 to T-127; M-1 to G-126; M-1 to E-125; M-1 to G-124; M-1 to C-123; M-1 to K-122; M-1 to S-121; M-1 to C-120; M-1 to V-119; M-1 to Q-118; M-1 to N-117; M-1 to K-116; M-1 to M-115; M-1 to E-114; M-1 to L-113; M-1 to Y-112; M-1 to E-111; M-1 to G-110; M-1 to S-109; M-1 to A-108; M-1 to C-107; M-1 to S-106; M-1 to F-105; M-1 to T-104; M-1 to C-103; M-1 to E-102; M-1 to K-101; M-1 to G-100; M-1 to R-99; M-1 to V-98; M-1 to P-97; M-1 to D-96; M-1 to P-95; M-1 to L-94; M-1 to G-93; M-1 to S-92; M-1 to C-91; M-1 to D-90; M-1 to V-89; M-1 to A-88; M-1 to S-87; M-1 to N-86; M-1 to P-85; M-1 to I-84; M-1 to A-83; M-1 to V-82; M-1 to R-81; M-1 to W-80; M-1 to R-79; M-1 to S-78; M-1 to G-77; M-1 to S-76; M-1 to S-75; M-1 to D-74; M-1 to C-73; M-1 to E-72; M-1 to T-71; M-1 to Y-70; M-1 to E-69; M-1 to F-68; M-1 to H-67; M-1 to Y-66; M-1 to D-65; M-1 to K-64; M-1 to E-63; M-1 to Q-62; M-1 to C-61; M-1 to P-60; M-1 to P-59; M-1 to L-58; M-1 to P-57; M-1 to R-56; M-1 to S-55; M-1 to S-54; M-1 to S-53; M-1 to S-52; M-1 to P-51; M-1 to L-50; M-1 to D-49; M-1 to G-48; M-1 to A-47; M-1 to W-46; M-1 to A-45; M-1 to A-44; M-1 to Q-43; M-1 to C-42; M-1 to G-41; M-1 to A-40; M-1 to L-39; M-1 to A-38; M-1 to W-37; M-1 to C-36; M-1 to C-35; M-1 to I-34; M-1 to W-33; M-1 to A-32; M-1 to P-31; M-1 to S-30; M-1 to W-29; M-1 to P-28; M-1 to P-27; M-1 to S-26; M-1 to R-25; M-1 to G-24; M-1 to R-23; M-1 to R-22; M-1 to P-21; M-1 to A-20; M-1 to E-19; M-1 to A-18; M-1 to P-17; M-1 to R-16; M-1 to G-15; M-1 to W-14; M-1 to G-13; M-1 to R-12; M-1 to G-11; M-1 to R-10; M-1 to V-9; M-1 to P-8; M-1 to G-7; and/or M-1 to R-6 of the TR16-short sequence shown in Figures 1A-E (SEQ ID NO:2). The present invention is also directed to nucleic acid molecules comprising or, alternatively, consisting of a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide

sequences encoding the TR16 polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0157] Additionally, the present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the TR16-long polypeptide shown in Figures 4A-E (SEQ ID NO:4), up to the arginine residue at position number 6, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues 1-m³ of Figures 4A-E (SEQ ID NO:4), where m³ is an integer from 6 to 1027 corresponding to the position of the amino acid residue in Figures 4A-E (SEQ ID NO:4).

[0158] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues: M-1 to N-1026; M-1 to P-1025; M-1 to S-1024; M-1 to R-1023; M-1 to S-1022; M-1 to T-1021; M-1 to K-1020; M-1 to L-1019; M-1 to Q-1018; M-1 to V-1017; M-1 to S-1016; M-1 to E-1015; M-1 to F-1014; M-1 to H-1013; M-1 to D-1012; M-1 to E-1011; M-1 to K-1010; M-1 to E-1009; M-1 to K-1008; M-1 to T-1007; M-1 to A-1006; M-1 to L-1005; M-1 to S-1004; M-1 to K-1003; M-1 to L-1002; M-1 to K-1001; M-1 to G-1000; M-1 to L-999; M-1 to L-998; M-1 to S-997; M-1 to Q-996; M-1 to K-995; M-1 to N-994; M-1 to S-993; M-1 to Y-992; M-1 to V-991; M-1 to V-990; M-1 to E-989; M-1 to E-988; M-1 to E-987; M-1 to N-986; M-1 to D-985; M-1 to E-984; M-1 to G-983; M-1 to E-982; M-1 to M-981; M-1 to I-980; M-1 to A-979; M-1 to C-978; M-1 to S-977; M-1 to D-976; M-1 to A-975; M-1 to A-974; M-1 to P-973; M-1 to L-972; M-1 to E-971; M-1 to C-970; M-1 to E-969; M-1 to K-968; M-1 to S-967; M-1 to N-966; M-1 to T-965; M-1 to T-964; M-1 to M-963; M-1 to V-962; M-1 to L-961; M-1 to K-960; M-1 to S-959; M-1 to Y-958; M-1 to K-957; M-1 to Y-956; M-1 to E-955; M-1 to L-954; M-1 to K-953; M-1 to Q-952; M-1 to N-951; M-1 to K-950; and/or M-1 to K-949; of the TR16-long sequence shown in Figures 4A-E (SEQ ID NO:4). The present invention is also directed to nucleic acid molecules comprising or, alternatively, consisting of a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequences encoding the TR16 polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide

sequence. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0159] The present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the mature TR16-short polypeptide shown in Figures 1A-E, up to the serine residue at position number 53, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues 48-m² of Figures 1A-E, where m² is an integer from 53 to 962 corresponding to the position of the amino acid residue in Figures 1A-E (SEQ ID NO:2).

[0160] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues G-48 to F-962; G-48 to L-961; G-48 to N-960; G-48 to L-959; G-48 to I-958; G-48 to T-957; G-48 to K-956; G-48 to K-955; G-48 to K-954; G-48 to K-953; G-48 to Q-952; G-48 to N-951; G-48 to K-950; G-48 to K-949; G-48 to W-948; G-48 to F-947; G-48 to Y-946; G-48 to C-945; G-48 to T-944; G-48 to L-943; G-48 to A-942; G-48 to V-941; G-48 to L-940; G-48 to L-939; G-48 to V-938; G-48 to A-937; G-48 to T-936; G-48 to F-935; G-48 to A-934; G-48 to G-933; G-48 to V-932; G-48 to G-931; G-48 to A-930; G-48 to G-929; G-48 to V-928; G-48 to K-927; G-48 to L-926; G-48 to W-925; G-48 to F-924; G-48 to D-923; G-48 to V-922; G-48 to T-921; G-48 to E-920; G-48 to C-919; G-48 to T-918; G-48 to A-917; G-48 to L-916; G-48 to K-915; G-48 to K-914; G-48 to E-913; G-48 to P-912; G-48 to L-911; G-48 to S-910; G-48 to I-909; G-48 to G-908; G-48 to K-907; G-48 to I-906; G-48 to C-905; G-48 to W-904; G-48 to K-903; G-48 to P-902; G-48 to E-901; G-48 to N-900; G-48 to W-899; G-48 to V-898; G-48 to Y-897; G-48 to L-896; G-48 to T-895; G-48 to E-894; G-48 to Q-893; G-48 to F-892; G-48 to G-891; G-48 to R-890; G-48 to K-889; G-48 to C-888; G-48 to A-887; G-48 to G-886; G-48 to E-885; G-48 to I-884; G-48 to E-883; G-48 to H-882; G-48 to F-881; G-48 to D-880; G-48 to H-879; G-48 to E-878; G-48 to T-877; G-48 to C-876; G-48 to L-875; G-48 to P-874; G-48 to C-873; G-48 to A-872; G-48 to E-871; G-48 to A-870; G-48 to S-869; G-48 to E-868; G-48 to W-867; G-48 to L-866; G-48 to F-865; G-48 to Y-864; G-48 to F-863; G-48 to T-862; G-48 to C-861; G-48 to G-860; G-48 to D-859; G-48 to C-858; G-48 to T-857; G-48 to G-856; G-48 to A-855; G-48 to P-854; G-48 to C-853; G-48 to K-852; G-48 to S-851; G-48 to P-850; G-48 to V-849; G-48 to S-848; G-48 to I-847; G-48 to V-846; G-48 to G-845; G-48

to A-844; G-48 to G-843; G-48 to S-842; G-48 to K-841; G-48 to T-840; G-48 to P-839; G-48 to N-838; G-48 to C-837; G-48 to R-836; G-48 to M-835; G-48 to K-834; G-48 to V-833; G-48 to A-832; G-48 to T-831; G-48 to S-830; G-48 to R-829; G-48 to G-828; G-48 to N-827; G-48 to I-826; G-48 to C-825; G-48 to S-824; G-48 to T-823; G-48 to T-822; G-48 to A-821; G-48 to T-820; G-48 to S-819; G-48 to S-818; G-48 to K-817; G-48 to Y-816; G-48 to F-815; G-48 to F-814; G-48 to H-813; G-48 to V-812; G-48 to D-811; G-48 to P-810; G-48 to I-809; G-48 to Q-808; G-48 to S-807; G-48 to T-806; G-48 to P-805; G-48 to V-804; G-48 to P-803; G-48 to F-802; G-48 to M-801; G-48 to D-800; G-48 to E-799; G-48 to K-798; G-48 to I-797; G-48 to N-796; G-48 to I-795; G-48 to N-794; G-48 to K-793; G-48 to L-792; G-48 to T-791; G-48 to T-790; G-48 to E-789; G-48 to V-788; G-48 to T-787; G-48 to V-786; G-48 to G-785; G-48 to I-784; G-48 to F-783; G-48 to T-782; G-48 to D-781; G-48 to A-780; G-48 to L-779; G-48 to I-778; G-48 to I-777; G-48 to S-776; G-48 to Q-775; G-48 to S-774; G-48 to S-773; G-48 to L-772; G-48 to A-771; G-48 to A-770; G-48 to R-769; G-48 to F-768; G-48 to G-767; G-48 to K-766; G-48 to S-765; G-48 to E-764; G-48 to S-763; G-48 to P-762; G-48 to I-761; G-48 to I-760; G-48 to T-759; G-48 to S-758; G-48 to Q-757; G-48 to C-756; G-48 to V-755; G-48 to F-754; G-48 to A-753; G-48 to G-752; G-48 to V-751; G-48 to L-750; G-48 to N-749; G-48 to T-748; G-48 to Y-747; G-48 to D-746; G-48 to D-745; G-48 to S-744; G-48 to G-743; G-48 to A-742; G-48 to V-741; G-48 to I-740; G-48 to E-739; G-48 to K-738; G-48 to V-737; G-48 to T-736; G-48 to F-735; G-48 to D-734; G-48 to T-733; G-48 to I-732; G-48 to N-731; G-48 to N-730; G-48 to T-729; G-48 to C-728; G-48 to L-727; G-48 to A-726; G-48 to M-725; G-48 to K-724; G-48 to K-723; G-48 to G-722; G-48 to E-721; G-48 to H-720; G-48 to G-719; G-48 to C-718; G-48 to L-717; G-48 to S-716; G-48 to I-715; G-48 to N-714; G-48 to F-713; G-48 to F-712; G-48 to H-711; G-48 to F-710; G-48 to Y-709; G-48 to K-708; G-48 to T-707; G-48 to G-706; G-48 to K-705; G-48 to S-704; G-48 to T-703; G-48 to F-702; G-48 to S-701; G-48 to P-700; G-48 to G-699; G-48 to N-698; G-48 to M-697; G-48 to L-696; G-48 to S-695; G-48 to G-694; G-48 to V-693; G-48 to S-692; G-48 to S-691; G-48 to L-690; G-48 to N-689; G-48 to S-688; G-48 to F-687; G-48 to D-686; G-48 to Y-685; G-48 to H-684; G-48 to L-683; G-48 to I-682; G-48 to Q-681; G-48 to N-680; G-48 to E-679; G-48 to K-678; G-48 to E-677; G-48 to H-676; G-48 to Y-675; G-48 to F-674; G-48 to F-673; G-48 to C-672; G-48 to D-671; G-48 to S-670; G-48 to Y-669; G-48 to C-668; G-48 to V-667; G-48 to S-666; G-48 to H-665; G-48 to D-664; G-48 to Q-

663; G-48 to N-662; G-48 to N-661; G-48 to K-660; G-48 to S-659; G-48 to G-658; G-48 to P-657; G-48 to G-656; G-48 to C-655; G-48 to P-654; G-48 to I-653; G-48 to C-652; G-48 to A-651; G-48 to E-650; G-48 to K-649; G-48 to G-648; G-48 to Y-647; G-48 to V-646; G-48 to Q-645; G-48 to H-644; G-48 to I-643; G-48 to S-642; G-48 to L-641; G-48 to Y-640; G-48 to T-639; G-48 to D-638; G-48 to P-637; G-48 to P-636; G-48 to C-635; G-48 to E-634; G-48 to K-633; G-48 to C-632; G-48 to Q-631; G-48 to N-630; G-48 to T-629; G-48 to E-628; G-48 to K-627; G-48 to E-626; G-48 to I-625; G-48 to Y-624; G-48 to H-623; G-48 to G-622; G-48 to P-621; G-48 to P-620; G-48 to C-619; G-48 to P-618; G-48 to V-617; G-48 to C-616; G-48 to S-615; G-48 to S-614; G-48 to G-613; G-48 to S-612; G-48 to Q-611; G-48 to E-610; G-48 to S-609; G-48 to G-608; G-48 to L-607; G-48 to A-606; G-48 to C-605; G-48 to A-604; G-48 to R-603; G-48 to C-602; G-48 to S-601; G-48 to S-600; G-48 to A-599; G-48 to V-598; G-48 to G-597; G-48 to D-596; G-48 to V-595; G-48 to A-594; G-48 to N-593; G-48 to T-592; G-48 to A-591; G-48 to T-590; G-48 to I-589; G-48 to S-588; G-48 to Y-587; G-48 to I-586; G-48 to K-585; G-48 to V-584; G-48 to M-583; G-48 to D-582; G-48 to N-581; G-48 to I-580; G-48 to F-579; G-48 to R-578; G-48 to R-577; G-48 to N-576; G-48 to D-575; G-48 to Q-574; G-48 to G-573; G-48 to Q-572; G-48 to N-571; G-48 to T-570; G-48 to R-569; G-48 to Q-568; G-48 to F-567; G-48 to A-566; G-48 to W-565; G-48 to T-564; G-48 to F-563; G-48 to T-562; G-48 to F-561; G-48 to T-560; G-48 to A-559; G-48 to N-558; G-48 to K-557; G-48 to F-556; G-48 to I-555; G-48 to I-554; G-48 to H-553; G-48 to T-552; G-48 to Y-551; G-48 to A-550; G-48 to Q-549; G-48 to K-548; G-48 to E-547; G-48 to K-546; G-48 to T-545; G-48 to G-544; G-48 to G-543; G-48 to W-542; G-48 to S-541; G-48 to E-540; G-48 to V-539; G-48 to V-538; G-48 to N-537; G-48 to T-536; G-48 to S-535; G-48 to K-534; G-48 to R-533; G-48 to N-532; G-48 to I-531; G-48 to D-530; G-48 to V-529; G-48 to M-528; G-48 to F-527; G-48 to Y-526; G-48 to L-525; G-48 to V-524; G-48 to C-523; G-48 to D-522; G-48 to A-521; G-48 to S-520; G-48 to C-519; G-48 to L-518; G-48 to T-517; G-48 to E-516; G-48 to F-515; G-48 to V-514; G-48 to F-513; G-48 to T-512; G-48 to I-511; G-48 to R-510; G-48 to G-509; G-48 to L-508; G-48 to E-507; G-48 to S-506; G-48 to G-505; G-48 to T-504; G-48 to A-503; G-48 to G-502; G-48 to T-501; G-48 to M-500; G-48 to S-499; G-48 to T-498; G-48 to P-497; G-48 to P-496; G-48 to K-495; G-48 to F-494; G-48 to G-493; G-48 to P-492; G-48 to I-491; G-48 to H-490; G-48 to L-489; G-48 to N-488; G-48 to L-487; G-48 to I-486; G-48 to L-485; G-48 to Y-484; G-48 to D-483; G-48 to N-482;

G-48 to D-481; G-48 to S-480; G-48 to G-479; G-48 to G-478; G-48 to A-477; G-48 to G-476; G-48 to S-475; G-48 to Q-474; G-48 to I-473; G-48 to H-472; G-48 to D-471; G-48 to G-470; G-48 to A-469; G-48 to V-468; G-48 to E-467; G-48 to W-466; G-48 to G-465; G-48 to N-464; G-48 to M-463; G-48 to G-462; G-48 to D-461; G-48 to C-460; G-48 to K-459; G-48 to S-458; G-48 to N-457; G-48 to G-456; G-48 to V-455; G-48 to N-454; G-48 to F-453; G-48 to C-452; G-48 to S-451; G-48 to T-450; G-48 to K-449; G-48 to M-448; G-48 to N-447; G-48 to G-446; G-48 to P-445; G-48 to L-444; G-48 to V-443; G-48 to N-442; G-48 to W-441; G-48 to W-440; G-48 to K-439; G-48 to Y-438; G-48 to E-437; G-48 to F-436; G-48 to G-435; G-48 to L-434; G-48 to A-433; G-48 to P-432; G-48 to E-431; G-48 to T-430; G-48 to G-429; G-48 to A-428; G-48 to P-427; G-48 to C-426; G-48 to P-425; G-48 to R-424; G-48 to C-423; G-48 to E-422; G-48 to K-421; G-48 to T-420; G-48 to G-419; G-48 to D-418; G-48 to S-417; G-48 to F-416; G-48 to T-415; G-48 to G-414; G-48 to P-413; G-48 to P-412; G-48 to C-411; G-48 to P-410; G-48 to H-409; G-48 to C-408; G-48 to S-407; G-48 to S-406; G-48 to S-405; G-48 to G-404; G-48 to N-403; G-48 to N-402; G-48 to Y-401; G-48 to F-400; G-48 to G-399; G-48 to P-398; G-48 to N-397; G-48 to C-396; G-48 to P-395; G-48 to P-394; G-48 to C-393; G-48 to D-392; G-48 to K-391; G-48 to K-390; G-48 to E-389; G-48 to G-388; G-48 to S-387; G-48 to P-386; G-48 to P-385; G-48 to L-384; G-48 to R-383; G-48 to I-382; G-48 to A-381; G-48 to D-380; G-48 to T-379; G-48 to L-378; G-48 to D-377; G-48 to E-376; G-48 to R-375; G-48 to C-374; G-48 to I-373; G-48 to K-372; G-48 to P-371; G-48 to E-370; G-48 to I-369; G-48 to W-368; G-48 to K-367; G-48 to Y-366; G-48 to M-365; G-48 to I-364; G-48 to Q-363; G-48 to T-362; G-48 to K-361; G-48 to G-360; G-48 to E-359; G-48 to E-358; G-48 to D-357; G-48 to C-356; G-48 to P-355; G-48 to T-354; G-48 to H-353; G-48 to I-352; G-48 to Q-351; G-48 to F-350; G-48 to Y-349; G-48 to D-348; G-48 to K-347; G-48 to T-346; G-48 to T-345; G-48 to C-344; G-48 to P-343; G-48 to P-342; G-48 to R-341; G-48 to E-340; G-48 to T-339; G-48 to C-338; G-48 to E-337; G-48 to S-336; G-48 to S-335; G-48 to G-334; G-48 to S-333; G-48 to F-332; G-48 to Q-331; G-48 to S-330; G-48 to D-329; G-48 to D-328; G-48 to K-327; G-48 to C-326; G-48 to R-325; G-48 to I-324; G-48 to C-323; G-48 to E-322; G-48 to K-321; G-48 to A-320; G-48 to G-319; G-48 to K-318; G-48 to E-317; G-48 to S-316; G-48 to Y-315; G-48 to T-314; G-48 to N-313; G-48 to R-312; G-48 to P-311; G-48 to C-310; G-48 to V-309; G-48 to Q-308; G-48 to C-307; G-48 to N-306; G-48 to F-305; G-48 to S-304; G-48 to G-303; G-48 to P-302; G-48 to K-301;

G-48 to N-300; G-48 to S-299; G-48 to F-298; G-48 to T-297; G-48 to G-296; G-48 to P-295; G-48 to K-294; G-48 to C-293; G-48 to P-292; G-48 to F-291; G-48 to C-290; G-48 to E-289; G-48 to S-288; G-48 to T-287; G-48 to Y-286; G-48 to A-285; G-48 to V-284; G-48 to G-283; G-48 to E-282; G-48 to I-281; G-48 to T-280; G-48 to I-279; G-48 to N-278; G-48 to K-277; G-48 to V-276; G-48 to L-275; G-48 to V-274; G-48 to P-273; G-48 to K-272; G-48 to V-271; G-48 to A-270; G-48 to K-269; G-48 to S-268; G-48 to G-267; G-48 to M-266; G-48 to L-265; G-48 to I-264; G-48 to G-263; G-48 to T-262; G-48 to T-261; G-48 to R-260; G-48 to W-259; G-48 to Y-258; G-48 to L-257; G-48 to I-256; G-48 to N-255; G-48 to T-254; G-48 to G-253; G-48 to S-252; G-48 to K-251; G-48 to L-250; G-48 to M-249; G-48 to V-248; G-48 to S-247; G-48 to H-246; G-48 to S-245; G-48 to G-244; G-48 to W-243; G-48 to E-242; G-48 to G-241; G-48 to N-240; G-48 to D-239; G-48 to T-238; G-48 to L-237; G-48 to K-236; G-48 to V-235; G-48 to W-234; G-48 to K-233; G-48 to D-232; G-48 to T-231; G-48 to T-230; G-48 to T-229; G-48 to D-228; G-48 to M-227; G-48 to E-226; G-48 to Q-225; G-48 to C-224; G-48 to Q-223; G-48 to D-222; G-48 to N-221; G-48 to Q-220; G-48 to I-219; G-48 to F-218; G-48 to F-217; G-48 to E-216; G-48 to F-215; G-48 to F-214; G-48 to I-213; G-48 to N-212; G-48 to N-211; G-48 to D-210; G-48 to V-209; G-48 to Y-208; G-48 to Q-207; G-48 to Y-206; G-48 to E-205; G-48 to F-204; G-48 to F-203; G-48 to V-202; G-48 to Y-201; G-48 to G-200; G-48 to S-199; G-48 to K-198; G-48 to K-197; G-48 to L-196; G-48 to H-195; G-48 to V-194; G-48 to A-193; G-48 to Y-192; G-48 to I-191; G-48 to L-190; G-48 to S-189; G-48 to V-188; G-48 to T-187; G-48 to C-186; G-48 to D-185; G-48 to D-184; G-48 to R-183; G-48 to N-182; G-48 to S-181; G-48 to E-180; G-48 to I-179; G-48 to Y-178; G-48 to N-177; G-48 to G-176; G-48 to R-175; G-48 to P-174; G-48 to I-173; G-48 to W-172; G-48 to S-171; G-48 to S-170; G-48 to N-169; G-48 to N-168; G-48 to C-167; G-48 to G-166; G-48 to D-165; G-48 to P-164; G-48 to R-163; G-48 to S-162; G-48 to D-161; G-48 to S-160; G-48 to P-159; G-48 to G-158; G-48 to V-157; G-48 to V-156; G-48 to T-155; G-48 to D-154; G-48 to M-153; G-48 to F-152; G-48 to T-151; G-48 to A-150; G-48 to I-149; G-48 to N-148; G-48 to S-147; G-48 to F-146; G-48 to G-145; G-48 to A-144; G-48 to P-143; G-48 to L-142; G-48 to E-141; G-48 to D-140; G-48 to W-139; G-48 to E-138; G-48 to D-137; G-48 to F-136; G-48 to K-135; G-48 to I-134; G-48 to G-133; G-48 to S-132; G-48 to G-131; G-48 to L-130; G-48 to S-129; G-48 to Y-128; G-48 to T-127; G-48 to G-126; G-48 to E-125; G-48 to G-124; G-48 to C-123; G-48 to K-122; G-48 to S-121; G-48 to C-120; G-48

to V-119; G-48 to Q-118; G-48 to N-117; G-48 to K-116; G-48 to M-115; G-48 to E-114; G-48 to L-113; G-48 to Y-112; G-48 to E-111; G-48 to G-110; G-48 to S-109; G-48 to A-108; G-48 to C-107; G-48 to S-106; G-48 to F-105; G-48 to T-104; G-48 to C-103; G-48 to E-102; G-48 to K-101; G-48 to G-100; G-48 to R-99; G-48 to V-98; G-48 to P-97; G-48 to D-96; G-48 to P-95; G-48 to L-94; G-48 to G-93; G-48 to S-92; G-48 to C-91; G-48 to D-90; G-48 to V-89; G-48 to A-88; G-48 to S-87; G-48 to N-86; G-48 to P-85; G-48 to I-84; G-48 to A-83; G-48 to V-82; G-48 to R-81; G-48 to W-80; G-48 to R-79; G-48 to S-78; G-48 to G-77; G-48 to S-76; G-48 to S-75; G-48 to D-74; G-48 to C-73; G-48 to E-72; G-48 to T-71; G-48 to Y-70; G-48 to E-69; G-48 to F-68; G-48 to H-67; G-48 to Y-66; G-48 to D-65; G-48 to K-64; G-48 to E-63; G-48 to Q-62; G-48 to C-61; G-48 to P-60; G-48 to P-59; G-48 to L-58; G-48 to P-57; G-48 to R-56; G-48 to S-55; G-48 to S-54; and/or G-48 to S-53 of the mature TR16-short sequence shown in Figures 1A-E (SEQ ID NO:2). The present invention is also directed to nucleic acid molecules comprising or, alternatively, consisting of a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequences encoding the TR16 polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0161] The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues n^1 - m^1 , n^1 - m^2 , n^2 - m^1 , and/or n^2 - m^2 of Figures 1A-E (i.e., SEQ ID NO:2) or n^3 - m^3 of Figures 4A-E (SEQ ID NO:4) where n^1 , n^2 , n^3 , m^1 , m^2 , and m^3 are integers as described above. Thus, any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted TR16 polypeptide.

[0162] In a specific embodiment, any of the above N- and/or C-terminal deleted TR16 polypeptides is fused with the polypeptide sequence MAPWNVLPGPFPFHSSRL HGSGHSRLAAAAISIALKAFSCASG (SEQ ID NO:29).

[0163] It will be recognized in the art that some amino acid sequences of TR16 can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Thus, the invention further includes variations of the TR16 receptor, which show substantial TR16 receptor activity or which

include regions of TR16 proteins, such as the protein portions discussed herein. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in J.U. Bowie *et al.*, *Science* 247:1306-1310 (1990).

[0164] Thus, the fragment, derivative, or analog of the polypeptide of Figures 1A-E (SEQ ID NO:2), or the polypeptide of Figures 4A-E (SEQ ID NO:4), or a polypeptide encoded by one of the deposited cDNAs, may be (i) one in which at least one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue(s), and more preferably at least one but less than ten conserved amino acid residues) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

[0165] Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the TR16 receptor protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al.*, *Clin Exp. Immunol.* 2:331-340 (1967); Robbins *et al.*, *Diabetes* 36:838-845 (1987); Cleland *et al.* *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

[0166] The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al.*, *Nature* 361:266-268 (1993), describes certain mutations resulting in selective binding of TNF- α to only one of the two known types of TNF receptors. Thus, the TR16 polypeptides of the present invention may include one or more amino acid substitutions, deletions, or additions, either from natural mutations or human manipulation.

[0167] As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table III).

TABLE III.
Conservative Amino Acid Substitutions

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

[0168] In specific embodiments, the number of substitutions, additions or deletions in the amino acid sequence of Figures 1A-E and/or Figures 4A-E (SEQ ID NO:4) and/or any of the polypeptide fragments described herein (e.g., the extracellular domain or intracellular domain) is 75, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 30-20, 20-15, 20-10, 15-10, 10-1, 5-10, 1-5, 1-3 or 1-2.

[0169] Amino acids in the TR16 proteins of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis

or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.* 224:899-904 (1992) and de Vos *et al.* *Science* 255:306-312 (1992)).

[0170] To improve or alter the characteristics of TR16 polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

[0171] Non-naturally occurring variants may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (see e.g., Carter *et al.*, *Nucl. Acids Res.* 13:4331 (1986); and Zoller *et al.*, *Nucl. Acids Res.* 10:6487 (1982)), cassette mutagenesis (see e.g., Wells *et al.*, *Gene* 34:315 (1985)), restriction selection mutagenesis (see e.g., Wells *et al.*, *Philos. Trans. R. Soc. London SerA* 317:415 (1986)).

[0172] Thus, the invention also encompasses TR16 derivatives and analogs that have one or more amino acid residues deleted, added, or substituted to generate TR16 polypeptides that are better suited for expression, scale up, etc., in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions on any one or more of the glycosylation recognition sequences in the TR16 polypeptides of the invention, and/or an amino acid deletion at the second position of any one or more such recognition sequences will prevent glycosylation of the

TR16 at the modified tripeptide sequence (see, e.g., Miyajimo *et al.*, *EMBO J* 5(6):1193-1197). Additionally, one or more of the amino acid residues of the polypeptides of the invention (e.g., arginine and lysine residues) may be deleted or substituted with another residue to eliminate undesired processing by proteases such as, for example, furins or kexins.

[0173] The polypeptides of the present invention include a polypeptide comprising, or alternatively, consisting of a polypeptide encoded by one of the deposited cDNAs including the leader; a polypeptide comprising, or alternatively, consisting of a mature polypeptide sequence encoded by one of the deposited cDNAs minus the leader (i.e., the mature protein); a polypeptide comprising, or alternatively, consisting of amino acids from about 1 to about 963 in Figures 1A-E (SEQ ID NO:2); a polypeptide comprising, or alternatively, consisting of amino acids from about 1 to about 1027 in Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively, consisting of amino acids from about 2 to about 963 in Figures 1A-E (SEQ ID NO:2); a polypeptide comprising, or alternatively, consisting of amino acids from about 2 to about 1027 in Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively, consisting of amino acids from about 48 to about 963 in Figures 1A-E (SEQ ID NO:2); a polypeptide comprising, or alternatively, consisting of amino acids from about 48 to about 1027 in Figures 4A-E (SEQ ID NO:4); a polypeptide comprising, or alternatively, consisting of the TR16 extracellular domain; a polypeptide comprising, or alternatively, consisting of the TR16 cysteine rich domain; a polypeptide comprising, or alternatively, consisting of the TR16 transmembrane domain; a polypeptide comprising, or alternatively, consisting of the intracellular domain of TR16-short; a polypeptide comprising, or alternatively, consisting of the intracellular domain of TR16-long; and a polypeptide comprising, or alternatively, consisting of the TR16 extracellular domain and one of the TR16 intracellular domains with all or part of the transmembrane domain deleted; as well as polypeptides which are at least 80% or 85% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98%, 99% or 100% identical to the polypeptides described above (e.g., the polypeptide encoded by one of the deposited cDNA clones, the polypeptide of Figures 1A-E (SEQ ID NO:2), and the polypeptide of Figures 4A-E (SEQ ID NO:4)), and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 or at least 100 amino acids. Polynucleotides encoding these

polypeptides are also encompassed by the invention.

[0174] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a TR16 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the TR16 receptor. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0175] As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to, for instance, the amino acid sequence shown in Figures 1A-E (SEQ ID NO:2), or to the amino acid sequence shown in Figures 4A-E (SEQ ID NO:4), or to an amino acid sequence encoded by one of the deposited cDNA clones, can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

[0176] In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (*Comp. App. Biosci.* 6:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2,

Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the

FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

[0177] In additional embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the polynucleotide sequence encoding one or more of the extracellular cysteine rich motifs of TR16 disclosed in Figures 1A-E and Figures 4A-E (amino acid residues from about 290 to 344, 356 to 426, 602 to 672, and/or 825 to 919). In another embodiment, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to DNA complementary to the polynucleotide sequence encoding one, two, three, or four of the TR16 extracellular cysteine rich motifs. The present invention also encompasses the above polynucleotide/nucleic acid sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention.

[0178] The present application is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the TR16 polypeptide sequence set forth as n^1 - m^1 , and/or n^2 - m^1 herein. In preferred embodiments, the application is directed to proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific TR16 N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0179] In certain preferred embodiments, TR16 proteins of the invention comprise fusion proteins as described above wherein the TR16 polypeptides are those described as n^1 - m^1 , and/or n^2 - m^1 herein. In preferred embodiments, the application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0180] In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide described herein. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody

response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998- 4002 (1983).

[0181] As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, J.G. Sutcliffe *et al.*, "Antibodies That React With Predetermined Sites on Proteins," *Science* 219:660-666 (1983). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immuno-dominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

[0182] Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful, for example, to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al.*, *Cell* 37:767-778 (1984) at 777. Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least four, at least five, at least six, at least seven, more preferably at least nine, at least 20, at least 25, at least 30, at least 40, at least 50 and most preferably between at least about 55 to about 100 amino acids contained within the amino acid sequence of a polypeptide of the invention. Non-limiting examples of antigenic polypeptides or peptides that can be used to generate TR16 receptor-specific antibodies include: a polypeptide comprising or alternatively consisting of amino acid residues from about 51 to about 67 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 72 to about 79 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 94 to about 104 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 159 to about 171 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E

(SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 180 to about 185 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 222 to about 233 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 238 to about 242 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 313 to about 319 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 325 to about 348 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 355 to about 362 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 385 to about 395 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 418 to about 430 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 456 to about 465 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 479 to about 483 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 530 to about 535 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 543 to about 548 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 569 to about 579 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 608 to about 615 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 627 to about 639 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 658 to about 665 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E

(SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 702 to about 707 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 719 to about 724 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 744 to about 747 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 763 to about 767 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 837 to about 842 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 849 to about 856 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); a polypeptide comprising or alternatively consisting of amino acid residues from about 886 to about 813 in Figures 1A-E (SEQ ID NO:2) or Figures 4A-E (SEQ ID NO:4); and/or a polypeptide comprising or alternatively consisting of amino acid residues from about 950 to about 955 in Figures 1A-E (SEQ ID NO:2). As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the TR16 receptor protein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0183] The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. R.A. Houghten, "General Method for the Rapid Solid-phase Synthesis of Large Numbers of Peptides: Specificity of Antigen-Antibody Interaction at the Level of Individual Amino Acids," *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

[0184] As one of skill in the art will appreciate, TR16 receptor polypeptides of the present invention and the epitope-bearing fragments thereof, described herein (e.g., corresponding to a portion of the extracellular domain, such as, for example, amino acid residues 1 to 923 of SEQ ID NO:2 or Figures 4A-E), can be combined with heterologous polypeptide sequences, for example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and

portions thereof), resulting in chimeric polypeptides. IgG molecules and portions thereof (e.g., Fc fragments) that may be used to produce fusion proteins in accordance with the invention include each of the four subclasses of human IgG: IgG1, IgG2, IgG3, and IgG4. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al.*, *Nature* 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric TR16 protein or protein fragment alone (Fountoulakis *et al.*, *J. Biochem.* 270:3958-3964 (1995)).

[0185] Preferred Fc fusions of the present invention include, but are not limited to constructs comprising, or alternatively consisting of, amino acid residues 1 to 923, 1 to 915, 10 to 923, 20 to 923, 40 to 923, 44 to 923, 48 to 923, 48 to 920, 48 to 917, and/or 10 to 140 of Figures 1A-E (SEQ ID NO:2).

[0186] Preferred Fc fusions of the present invention include, but are not limited to constructs comprising, or alternatively consisting of, amino acid residues 1 to 923, 1 to 915, 10 to 923, 20 to 923, 40 to 923, 44 to 923, 48 to 923, 48 to 920, 48 to 917, and/or 10 to 140 of Figures 4A-E (SEQ ID NO:4).

[0187] The polypeptides of the present invention have uses which include, but are not limited to, as sources for generating antibodies that bind the polypeptides of the invention, and as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

DIAGNOSTIC ASSAYS

[0188] The compounds of the present invention are useful for diagnosis or treatment of various immune system-related disorders in mammals, preferably humans. Such disorders include but are not limited to tumors (e.g., B cell and monocytic cell leukemias and lymphomas) and tumor metastasis, infections by bacteria, viruses and other parasites, immunodeficiencies, inflammatory diseases, lymphadenopathy, autoimmune diseases, and graft versus host disease.

[0189] TR16 is expressed in B cells and spleen. For a number of immune

system-related disorders, substantially altered (increased or decreased) levels of TR16-short and/or TR16-long gene expression can be detected in immune system tissue or other cells or bodily fluids (e.g., sera, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" TR16-short and/or TR16-long gene expression level, that is, the TR16-short and/or TR16-long expression level in immune system tissues or bodily fluids from an individual not having the immune system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of an system disorder, which involves measuring the expression level of the gene encoding the TR16-short and/or TR16-long polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard TR16-short and/or TR16-long gene expression level, whereby an increase or decrease in the gene expression level(s) compared to the standard is indicative of an immune system disorder or normal activation, proliferation, differentiation, and/or death.

[0190] In particular, it is believed that certain tissues in mammals with cancer of cells or tissue of the immune system express significantly enhanced or reduced levels of normal or altered TR16-short and/or TR16-long polypeptide and mRNA encoding the TR16-short and/or TR16-long polypeptide when compared to a corresponding "standard" level. Further, it is believed that enhanced or depressed levels of the TR16-short and/or TR16-long polypeptide can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) or cells or tissue from mammals with such a cancer when compared to sera from mammals of the same species not having the cancer.

[0191] For example, as disclosed herein, TR16-short and/or TR16-long are expressed in B cells. Accordingly, polynucleotides of the invention (e.g., polynucleotide sequences complementary to all or a portion of TR16-short and/or TR16-long mRNA) and antibodies (and antibody fragments) directed against the polypeptides of the invention may be used to quantitate or qualitate concentrations of cells of B cell lineage (e.g., B cell leukemia cells) expressing TR16-short and/or TR16-long on their cell surfaces. These antibodies additionally have diagnostic applications in detecting abnormalities in the level of TR16-short and/or TR16-long gene expression, or abnormalities in the structure and/or temporal, tissue, cellular, or subcellular location of TR16-short and/or TR16-long. These diagnostic assays may be performed in vivo or in vitro, such as, for example, on blood samples, biopsy tissue or autopsy tissue.

[0192] Thus, the invention provides a diagnostic method useful during diagnosis of a immune system disorder, including cancers of this system, which involves measuring the expression level of the gene encoding the TR16-short and/or TR16-long polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard TR16-short and/or TR16-long gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder.

[0193] Where a diagnosis of a disorder in the immune system, including diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed TR16 and/or TR16-long gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

[0194] By "assaying the expression level of the gene encoding the TR16-short and/or TR16-long polypeptide" is intended qualitatively or quantitatively measuring or estimating the level of the TR16-short and/or TR16-long polypeptide or the level of the mRNA encoding the TR16-short and/or TR16-long polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the TR16-short and/or TR16-long polypeptide level or mRNA level in a second biological sample). Preferably, the TR16-short and/or TR16-long polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard TR16-short and/or TR16-long polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder of the immune system. As will be appreciated in the art, once a standard TR16-short and/or TR16-long polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[0195] By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source containing TR16 receptor protein (including portions thereof) or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain free extracellular domains of the TR16 polypeptide, immune system tissue, and other tissue sources found to express complete or free extracellular domain of the TR16 receptor.

Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0196] Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem.* 162:156-159 (1987). Levels of mRNA encoding the TR16-short and/or TR16-long polypeptide are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

[0197] The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of TR16-short and/or TR16-long receptor protein, or the soluble form thereof, in a biological sample (e.g., cells and tissues), including determination of normal and abnormal levels of polypeptides. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of TR16-short and/or TR16-long, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors, for example. Assay techniques that can be used to determine levels of a protein, such as a TR16-short and/or TR16-long protein of the present invention, or a soluble form thereof, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Assaying TR16-short and/or TR16-long protein levels in a biological sample can occur using any art-known method.

[0198] Assaying TR16-short and/or TR16-long polypeptide levels in a biological sample can occur using antibody-based techniques. For example, TR16-short and/or TR16-long polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al.*, *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, M., *et al.*, *J. Cell. Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting TR16-short and/or TR16-long polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include

enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium ($^{115\text{m}}\text{In}$, $^{113\text{m}}\text{In}$, ^{112}In , ^{111}In), and technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0199] The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the TR16 gene (such as, for example, cells of B cell lineage and the spleen) or cells or tissue which are known, or suspected, to express the TR16 ligand gene (such as, for example, cells of monocytic lineage). The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the TR16 gene or TR16 ligand gene.

[0200] For example, antibodies, or fragments of antibodies, such as those described herein, may be used to quantitatively or qualitatively detect the presence of TR16-short and/or TR16-long gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[0201] The antibodies (or fragments thereof), TR16 polypeptides, and/or TR16 ligands (e.g., Neutrokin- α) of the present invention may, additionally, be employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immunological assays, for in situ detection of TR16-short and/or TR16-long gene products or conserved variants or peptide fragments thereof, or for TR16 binding to TR16 ligand. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or TR16 polypeptide of the present invention. The antibody (or fragment) or TR16 polypeptide is preferably applied by

overlying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the TR16-short and/or TR16-long gene product, or conserved variants or peptide fragments, or TR16 polypeptide binding, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

[0202] Immunoassays and non-immunoassays for TR16-short and/or TR16-long gene products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled antibody capable of binding TR16-short and/or TR16-long gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

[0203] Immunoassays and non-immunoassays for TR16 ligand gene products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectable or labeled TR16 polypeptide capable of identifying TR16 ligand gene products or conserved variants or peptide fragments thereof, and detecting the bound TR16 polypeptide by any of a number of techniques well-known in the art.

[0204] The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled anti-TR16-short and/or anti-TR16-long antibody or detectable TR16-short and/or TR16-long polypeptide. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

[0205] By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene,

polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

[0206] The binding activity of a given lot of anti-TR16-short and/or anti-TR16-long antibody or TR16-short and/or TR16-long polypeptide may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0207] In addition to assaying TR16-short and/or TR16-long polypeptide levels or polynucleotide levels in a biological sample obtained from an individual, TR16-short and/or TR16-long polypeptide or polynucleotide can also be detected *in vivo* by imaging. For example, in one embodiment of the invention, TR16-short and/or TR16-long polypeptide is used to image monocytic leukemias or lymphomas. In another embodiment, TR16-short and/or TR16-long polynucleotides of the invention and/or anti-TR16 antibodies (e.g., polynucleotides complementary to all or a portion of TR16-short and/or TR16-long mRNA) are used to image B cell leukemias or lymphomas.

[0208] Antibody labels or markers for *in vivo* imaging of TR16-short and/or TR16-long polypeptide include those detectable by X-radiography, NMR, MRI, CAT-scans or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. Where *in vivo* imaging is used to detect enhanced levels of TR16-short and/or TR16-long polypeptide for diagnosis in humans, it may be preferable to use human antibodies or "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using techniques described herein or otherwise known in the art. For

example methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., *Nature* 312:643 (1984); Neuberger et al., *Nature* 314:268 (1985).

[0209] Additionally, any TR16-short and/or TR16-long polypeptide whose presence can be detected, can be administered. For example, TR16-short and/or TR16-long polypeptides labeled with a radio-opaque or other appropriate compound can be administered and visualized in vivo, as discussed, above for labeled antibodies. Further such TR16-short and/or TR16-long polypeptides can be utilized for in vitro diagnostic procedures.

[0210] A TR16-short and/or TR16-long polypeptide-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain TR16-short and/or TR16-long protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0211] With respect to antibodies, one of the ways in which the anti-TR16- short and/or anti-TR16-long antibody can be detectably labeled is by linking the same to an enzyme and using the linked product in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL.; Ishikawa,

E. et al., (eds.), 1981, Enzyme Immunoassay, Kigaku Shoin, Tokyo). The enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[0212] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect TR16- short and/or TR16-long through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

[0213] It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

[0214] The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu , or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0215] The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is

then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, therrromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0216] Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

TR16 BINDING PEPTIDES AND OTHER MOLECULES

[0217] The invention also encompasses screening methods for identifying polypeptide and non-polypeptide molecules that bind TR16, and the TR16 binding molecules identified thereby. These binding molecules are useful, for example, as agonists and antagonists of the TR16 receptor proteins. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

[0218] This method comprises the steps of:

contacting a TR16 protein or TR16-like protein with a plurality of molecules; and
identifying a molecule that binds the TR16 protein or TR16-like protein.

[0219] The step of contacting the TR16 protein or TR16-like protein with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing the TR16 protein or TR16-like protein on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized TR16 protein or TR16-like protein. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized TR16 protein or TR16-like protein. The molecules having a selective affinity for the TR16 protein or TR16-like protein can then be purified by affinity selection. The nature of the solid support, process for attachment of the TR16 protein or TR16-like protein to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

[0220] Alternatively, one may also separate a plurality of polypeptides into

substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by the TR16 protein or TR16-like protein, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the TR16 protein or TR16-like protein and the individual clone. Prior to contacting the TR16 protein or TR16-like protein with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for TR16 protein or TR16-like protein. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for the TR16 protein or TR16-like protein can be determined directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be determined more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be determined from the polypeptide itself, one may use micro-sequencing techniques. The sequencing technique may include mass spectroscopy.

[0221] In certain situations, it may be desirable to wash away any unbound TR16 protein or TR16-like protein, or alternatively, unbound polypeptides, from a mixture of the TR16 protein or TR16-like protein and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction. Such a wash step may be particularly desirable when the TR16 protein or TR16-like protein or the plurality of polypeptides is bound to a solid support.

[0222] The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or non-peptide libraries which can be screened for molecules that specifically bind to TR16. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and in vitro translation-based libraries.

Examples of chemically synthesized libraries are described in Fodor et al., 1991, Science 251:767-773; Houghten et al., 1991, Nature 354:84-86; Lam et al., 1991, Nature 354:82-84; Medynski, 1994, Bio/Technology 12:709-710; Gallop et al., 1994, J. Medicinal Chemistry 37(9):1233-1251; Ohlmeyer et al., 1993, Proc. Natl. Acad. Sci. USA 90:10922-10926; Erb et al., 1994, Proc. Natl. Acad. Sci. USA 91:11422-11426; Houghten et al., 1992, Biotechniques 13:412; Jayawickreme et al., 1994, Proc. Natl. Acad. Sci. USA 91:1614-1618; Salmon et al., 1993, Proc. Natl. Acad. Sci. USA 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381-5383.

[0223] Examples of phage display libraries are described in Scott and Smith, 1990, Science 249:386-390; Devlin et al., 1990, Science, 249:404-406; Christian, R. B., et al., 1992, J. Mol. Biol. 227:711-718; Lenstra, 1992, J. Immunol. Meth. 152:149-157; Kay et al., 1993, Gene 128:59-65; and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

[0224] In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Mattheakis et al., 1994, Proc. Natl. Acad. Sci. USA 91:9022-9026.

[0225] By way of examples of non-peptide libraries, a benzodiazepine library (see e.g., Bunin et al., 1994, Proc. Natl. Acad. Sci. USA 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, Proc. Natl. Acad. Sci. USA 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, Proc. Natl. Acad. Sci. USA 91:11138-11142).

[0226] The variety of non-peptide libraries that are useful in the present invention is great. For example, Ecker and Crooke, 1995, Bio/Technology 13:351-360 list benzodiazepines, hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

[0227] Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple

scaffold structure upon which a variety functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazepine structure.

[0228] Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added flexibility.

[0229] Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, Adv. Exp. Med. Biol. 251:215-218; Scott and Smith, 1990, Science 249:386-390; Fowlkes et al., 1992; BioTechniques 13:422-427; Oldenburg et al., 1992, Proc. Natl. Acad. Sci. USA 89:5393-5397; Yu et al., 1994, Cell 76:933-945; Staudt et al., 1988, Science 241:577-580; Bock et al., 1992, Nature 355:564-566; Tuerk et al., 1992, Proc. Natl. Acad. Sci. USA 89:6988-6992; Ellington et al., 1992, Nature 355:850-852; U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, Science 263:671-673; and CT Publication No. WO 94/18318.

[0230] In a specific embodiment, screening to identify a molecule that binds TR16 can be carried out by contacting the library members with a TR16 protein or TR16-like protein immobilized on a solid phase and harvesting those library members that bind to the TR16 protein or TR16-like protein. Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited herein.

[0231] In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields and Song, 1989, Nature 340:245-246; Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88:9578-9582) can be used to identify molecules that specifically bind to TR16 or TR16-like proteins.

[0232] Where the TR16 binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

[0233] Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine. Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries, such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

[0234] As mentioned above, in the case of a TR16 binding molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a TR16 binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

[0235] The selected TR16 binding polypeptide can be obtained by chemical synthesis or recombinant expression.

EPITOPES

[0236] The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence shown in Figures 1A-E or 4A-E, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in deposited clone HTWBD48 or HLICS62, contained in ATCC Deposit No. PTA-506, or encoded by a polynucleotide that hybridizes to the complement of the nucleotide sequence shown in Figures 1A-E or 4A-E or contained in deposited clone HTWBD48 or HLICS62, contained in ATCC Deposit No. PTA-506, under stringent hybridization conditions or lower stringency hybridization conditions as

defined *supra*. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence shown in Figures 1A-E or 4A-E), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined *supra*.

[0237] The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described *infra*. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross-reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

[0238] Exemplary epitopes are described in detail, above, and depicted in Figure 3 (as shown in tabular form in Table I, above) and in Figure 5 (as shown in tabular form in Table II, above).

[0239] Fragments that function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

[0240] In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Antigenic epitopes are useful, for example, to raise antibodies,

including monoclonal antibodies, that specifically bind the epitope. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., *Cell* 37:767-778 (1984); Sutcliffe et al., *Science* 219: 660-666 (1983)).

[0241] Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle et al., *J. Gen. Virol.* 66:2347-2354 (1985). The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as, for example, rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

[0242] Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, *in vitro* immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as, for example, rabbits, rats, and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 micrograms of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody that can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance,

by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

[0243] As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life *in vivo*. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix-binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

[0244] Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721;

5,834,252; and 5,837,458, and Patten et al., Curr. Opin. Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:1 and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide coding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

ANTIBODIES

[0245] The present invention further relates to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, preferably an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0246] Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and

fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

[0247] The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

[0248] Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention that they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies that specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

[0249] Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind

polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. Further included in the present invention are antibodies that bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or K_d less than $5 \times 10^{-2}M$, $10^{-2}M$, $5 \times 10^{-3}M$, $10^{-3}M$, $5 \times 10^{-4}M$, $10^{-4}M$, $5 \times 10^{-5}M$, $10^{-5}M$, $5 \times 10^{-6}M$, $10^{-6}M$, $5 \times 10^{-7}M$, $10^{-7}M$, $5 \times 10^{-8}M$, $10^{-8}M$, $5 \times 10^{-9}M$, $10^{-9}M$, $5 \times 10^{-10}M$, $10^{-10}M$, $5 \times 10^{-11}M$, $10^{-11}M$, $5 \times 10^{-12}M$, $10^{-12}M$, $5 \times 10^{-13}M$, $10^{-13}M$, $5 \times 10^{-14}M$, $10^{-14}M$, $5 \times 10^{-15}M$, and $10^{-15}M$.

[0250] The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0251] Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra).

In specific embodiments, antibodies are provided that inhibit ligand or receptor activity by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

[0252] The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., *Blood* 92(6):1981-1988 (1998); Chen, et al., *Cancer Res.* 58(16):3668-3678 (1998); Harrop et al., *J. Immunol.* 161(4):1786-1794 (1998); Zhu et al., *Cancer Res.* 58(15):3209-3214 (1998); Yoon, et al., *J. Immunol.* 160(7):3170-3179 (1998); Prat et al., *J. Cell. Sci.* 111(Pt2):237-247 (1998); Pitard et al., *J. Immunol. Methods* 205(2):177-190 (1997); Liautard et al., *Cytokine* 9(4):233-241 (1997); Carlson et al., *J. Biol. Chem.* 272(17):11295-11301 (1997); Taryman et al., *Neuron* 14(4):755-762 (1995); Muller et al., *Structure* 6(9):1153-1167 (1998); Bartunek et al., *Cytokine* 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

[0253] Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

[0254] As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

[0255] The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0256] The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

[0257] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0258] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well-known in the art and are described further in Example 5, below. Briefly, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[0259] Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

[0260] Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as

papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

[0261] For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0262] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better

et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

[0263] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entireties. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

[0264] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0265] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring that express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 96/34096; WO 96/33735; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; and 5,939,598, which are

incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0266] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

[0267] Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

POLYNUCLEOTIDES ENCODING ANTIBODIES

[0268] The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of Figures 1A-E or Figures 4A-E (SEQ ID NO:4).

[0269] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the

nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0270] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A⁺ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0271] Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0272] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to

determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

[0273] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. 81:851-855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

[0274] Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,694,778; Bird, 1988, Science 242:423-42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-54) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., 1988, Science 242:1038-1041).

METHODS OF PRODUCING ANTIBODIES

[0275] The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[0276] Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, e.g., a heavy or light chain of an antibody of the invention, requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[0277] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

1003-0000

[0278] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., 1986, *Gene* 45:101; Cockett et al., 1990, *Bio/Technology* 8:2).

[0279] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J.* 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is

produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0280] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0281] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:51-544).

[0282] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of

protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

[0283] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[0284] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1972, Proc. Natl. Acad. Sci. USA 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, Cell 22:817) genes can be employed in tk-, hgp^rt- or ap^rt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, Natl. Acad. Sci. USA 77:357; O'Hare et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance

to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIB TECH 11(5):155-215); and hygromycin (Santerre et al., 1984, Gene 30:147). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, Current Protocols in Human Genetics, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, J. Mol. Biol. 150:1, which are incorporated by reference herein in their entireties.

[0285] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

[0286] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52; Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0287] Once an antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by

affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

ANTIBODY CONJUGATES

[0288] The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20 or 50 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20 or 50 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

[0289] The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to

antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337-11341 (1992) (said references incorporated by reference in their entireties).

[0290] As discussed, supra, the polypeptides of the present invention may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides of the present invention may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988)). The polypeptides of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995)).

[0291] Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate their purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for

convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

[0292] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include iodine (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium ($^{115\text{m}}\text{In}$, $^{113\text{m}}\text{In}$, ^{112}In , ^{111}In), and technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru .

[0293] Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine,

propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0294] The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0295] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0296] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The

Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

[0297] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[0298] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

ASSAYS FOR ANTIBODY BINDING

[0299] The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[0300] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trayslol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot

analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

[0301] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

[0302] ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be

knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[0303] The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^3H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest is conjugated to a labeled compound (e.g., ^3H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

ANTIBODY-BASED THERAPEUTIC USES

[0304] The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the described disorders. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases and disorders associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to those diseases and disorders described in the section entitled "Therapeutics," below. The treatment and/or prevention of diseases and disorders associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases and disorders. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[0305] A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present

invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

[0306] The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

[0307] The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[0308] It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

ANTIBODY-BASED GENE THERAPY

[0309] In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of

the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0310] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0311] For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIBTECH* 11(5):155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; and Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY.

[0312] In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody nucleic acids (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

[0313] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

[0314] In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180 dated April 16, 1992 (Wu et al.); WO 92/22635 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Clarke et al.), WO 93/20221 dated October 14, 1993 (Young)). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

[0315] In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., 1993, Meth. Enzymol. 217:581-599). These retroviral vectors have been to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., 1994, Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more

resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, *J. Clin. Invest.* 93:644-651; Kiem et al., 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114.

[0316] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143- 155; Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234; PCT Publication WO94/12649; and Wang, et al., 1995, *Gene Therapy* 2:775-783. In a preferred embodiment, adenovirus vectors are used.

[0317] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300; U.S. Patent No. 5,436,146).

[0318] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0319] In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art

for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen et al., 1993, Meth. Enzymol. 217:618-644; Cline, 1985, Pharmac. Ther. 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[0320] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0321] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T-lymphocytes, B-lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0322] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[0323] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598, dated April 28, 1994; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

[0324] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such

that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

ANTIBODY-BASED DIAGNOSIS AND IMAGING

[0325] Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or disorders associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

[0326] The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0327] Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in

the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0328] One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of the interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[0329] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99}mTc . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

[0330] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12

hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0331] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disorder, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[0332] Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as positron emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0333] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

ANTIBODY-BASED KITS

[0334] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound,

an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[0335] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[0336] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[0337] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0338] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody

on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

[0339] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[0340] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC ACTIVITY

[0341] The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

THERAPEUTICS

[0342] The Tumor Necrosis Factor (TNF) family ligands are known to be among the

most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes (D.V. Goeddel *et al.*, "Tumor Necrosis Factors: Gene Structure and Biological Activities," *Symp. Quant. Biol.* 51:597- 609 (1986), Cold Spring Harbor; B. Beutler and A. Cerami, *Annu. Rev. Biochem.* 57:505-518 (1988); L.J. Old, *Sci. Am.* 258:59-75 (1988); W. Fiers, *FEBS Lett.* 285:199-224 (1991)). The TNF-family ligands induce such various cellular responses by binding to TNF-family receptors, including the TR16 of the present invention.

[0343] TR16 polynucleotides, polypeptides, agonists and/or antagonists of the invention may be administered to a patient (e.g., mammal, preferably human) afflicted with any disease or disorder mediated (directly or indirectly) by defective, or deficient levels of, TR16. Alternatively, a gene therapy approach may be applied to treat such diseases or disorders. In one embodiment of the invention, TR16 polynucleotide sequences are used to detect mutein TR16 genes, including defective genes. Mutein genes may be identified in *in vitro* diagnostic assays, and by comparison of the TR16 nucleotide sequence disclosed herein with that of a TR16 gene obtained from a patient suspected of harboring a defect in this gene. Defective genes may be replaced with normal TR16-encoding genes using techniques known to one skilled in the art.

[0344] In another embodiment, the TR16 polypeptides, polynucleotides, agonists and/or antagonists of the present invention are used as research tools for studying the phenotypic effects that result from inhibiting TR16/TR16 ligand interactions on various cell types. TR16 polypeptides and antagonists (e.g. monoclonal antibodies to TR16) also may be used in *in vitro* assays for detecting TR16, TR16 ligands, or the interactions thereof.

[0345] Cells or tissue which express the TR16 polypeptide and are believed to have a potent cellular response to TR16 ligands include B cells, spleen, brain, and testis. By "a cellular response to a TNF-family ligand" is intended any genotypic, phenotypic, and/or morphologic change to a cell, cell line, tissue, tissue culture or patient that is induced by a TNF-family ligand. As indicated, such cellular responses include not only normal physiological responses to TNF-family ligands, but also diseases associated dysregulation of these physiological responses, such as, for example, diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis-programmed cell death-is a

physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes (J.C. Ameisen, *AIDS* 8:1197-1213 (1994); P.H. Krammer *et al.*, *Curr. Opin. Immunol.* 6:279-289 (1994)).

[0346] Diseases associated with increased cell survival, or the inhibition of apoptosis, and that may be treated or prevented by the polynucleotides, polypeptides and/or agonists or antagonists of the invention include, but are not limited to, cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis rheumatoid arthritis); viral infections (such as herpes viruses, pox viruses and adenoviruses); inflammation; graft vs. host disease; acute graft rejection and chronic graft rejection. In preferred embodiments, TR16 polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above, or in the paragraph that follows.

[0347] Additional diseases or conditions associated with increased cell survival and that may be treated or prevented by the polynucleotides, polypeptides and/or agonists or antagonists of the invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), as well as large granular lymphocyte (LGL) leukemia, polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma,

endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

[0348] Thus, in preferred embodiments TR16 polynucleotides or polypeptides of the invention and agonists or antagonists thereof, are used to treat or prevent autoimmune diseases and/or inhibit the growth, progression, and/or metastasis of cancers, including, but not limited to, those cancers disclosed herein, such as, for example, lymphocytic leukemias (including, for example, MLL and chronic lymphocytic leukemia (CLL)) and follicular lymphomas. In another embodiment TR16 polynucleotides or polypeptides of the invention and/or agonists or antagonists thereof, are used to activate, differentiate or proliferate cancerous cells or tissue (e.g., B cell lineage related cancers (e.g., CLL and MLL), lymphocytic leukemia, or lymphoma) and thereby render the cells more vulnerable to cancer therapy (e.g., chemotherapy or radiation therapy).

[0349] Diseases associated with increased apoptosis and that may be treated or prevented by the polynucleotides, polypeptides and/or agonists or antagonists of the invention include, but are not limited to, AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis); myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (such as hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile

duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia. In preferred embodiments, TR16 polynucleotides, polypeptides and/or agonists are used to treat the diseases and disorders listed above.

[0350] Many of the pathologies associated with HIV are mediated by apoptosis, including HIV-induced nephropathy and HIV encephalitis. Thus, in additional preferred embodiments, TR16 polynucleotides, polypeptides, and/or TR16 agonists or antagonists of the invention are used to treat AIDS and pathologies associated with AIDS.

[0351] The state of immunodeficiency that defines AIDS is secondary to a decrease in the number and function of CD4⁺ T-lymphocytes. Recent reports estimate the daily loss of CD4⁺ T cells to be between 3.5×10^7 and 2×10^9 cells (Wei *et al.*, *Nature* 373:117-122 (1995)). One cause of CD4⁺ T cell depletion in the setting of HIV infection is believed to be HIV-induced apoptosis (see, for example, Meyaard *et al.*, *Science* 257:217-219, 1992; Groux *et al.*, *J Exp. Med.*, 175:331, 1992; and Oyaizu *et al.*, in *Cell Activation and Apoptosis in HIV Infection*, Andrieu and Lu, Eds., Plenum Press, New York, 1995, pp. 101-114). Indeed, HIV-induced apoptotic cell death has been demonstrated not only *in vitro* but also, more importantly, in infected individuals (J.C. Ameisen, *AIDS* 8:1197-1213 (1994); T.H. Finkel and N.K. Banda, *Curr. Opin. Immunol.* 6:605-615(1995); C.A. Muro-Cacho *et al.*, *J. Immunol.* 154:5555-5566 (1995)). Furthermore, apoptosis and CD4⁺ T-lymphocyte depletion is tightly correlated in different animal models of AIDS (T. Brunner *et al.*, *Nature* 373:441-444 (1995); M.L. Gougeon *et al.*, *AIDS Res. Hum. Retroviruses* 9:553-563 (1993)) and, apoptosis is not observed in those animal models in which viral replication does not result in AIDS. *Id.* Further data indicates that uninfected but primed or activated T lymphocytes from HIV-infected individuals undergo apoptosis after encountering the TNF-family ligand FasL. Using monocytic cell lines that result in death following HIV infection, it has been demonstrated that infection of U937 cells with HIV results in the *de novo* expression of FasL and that FasL mediates HIV-induced apoptosis (A.D. Badley *et al.*, *J. Virol.* 70:199-206 (1996)). Further, the TNF-family ligand was detectable in uninfected macrophages and its expression was upregulated following HIV infection resulting in selective killing of uninfected CD4 T-lymphocytes. *Id.* Thus, by the invention, a method for treating HIV⁺ individuals is provided which involves administering TR16 and/or TR16 agonists or antagonists of the present invention to reduce selective killing of CD4⁺ T-lymphocytes. Modes of

administration and dosages are discussed in detail below.

[0352] Activated human T cells are induced to undergo programmed cell death (apoptosis) upon triggering through the CD3/T cell receptor complex, a process termed activated-induced cell death (AICD). AICD of CD4⁺ T cells isolated from HIV-Infected asymptomatic individuals has been reported (Groux et al., *supra*). Thus, AICD may play a role in the depletion of CD4⁺ T cells and the progression to AIDS in HIV-infected individuals. Thus, the present invention provides a method of inhibiting TNF ligand-mediated T cell death in HIV patients, comprising administering a TR16 polypeptide of the invention (preferably, a soluble TR16 polypeptide) to the patients. In one embodiment, the patient is asymptomatic when treatment with TR16 commences. If desired, prior to treatment, peripheral blood T cells may be extracted from an HIV patient, and tested for susceptibility to TNF ligand-mediated cell death by procedures known in the art. In one embodiment, a patient's blood or plasma is contacted with TR16 *ex vivo*. The TR16 may be bound to a suitable chromatography matrix by procedures known in the art. The patient's blood or plasma flows through a chromatography column containing TR16 bound to the matrix, before being returned to the patient. The immobilized TR16 binds TNF ligand, thus removing TNF ligand protein from the patient's blood.

[0353] In additional embodiments a TR16 polypeptide of the invention is administered in combination with other inhibitors of T cell apoptosis. For example, Fas-mediated apoptosis and TRAIL-mediated apoptosis have also has been implicated in loss of T cells in HIV individuals (See, e.g., Katsikis *et al.*, *J. Exp. Med.* 181:2029-2036 (1995)). Thus, a patient susceptible to Fas ligand mediated and/or TRAIL mediated T cell death may be treated with an agent that blocks Fas-ligand/Fas receptor interactions and/or an agent that blocks TRAIL/TRAIL interactions.

[0354] Suitable agents for blocking binding of Fas-ligand to Fas that may be administered with the TR16 polynucleotides or polypeptides of the invention (including TR16 agonists and/or antagonists) include, but are not limited to, soluble Fas polypeptides; multimeric forms of soluble Fas polypeptides (e.g., dimers of sFas/Fc); anti-Fas antibodies that bind Fas without transducing the biological signal that results in apoptosis; anti-Fas-ligand antibodies that block binding of Fas-ligand to Fas; and muteins of Fas-ligand that bind Fas but do not transduce the biological signal that results in apoptosis. Preferably, the antibodies employed according to this method are monoclonal

antibodies. Examples of suitable agents for blocking Fas-ligand/Fas interactions, including blocking anti-Fas monoclonal antibodies, are described in International application publication number WO 95/10540, hereby incorporated by reference.

[0355] Suitable agents, which also block binding of TRAIL to a TRAIL receptor that may be administered with the polynucleotides and/or polypeptides of the present invention include, but are not limited to, soluble TRAIL receptor polypeptides (e.g., a soluble form of OPG, DR4 (International application publication number WO 98/32856); TR5 (International application publication number WO 98/30693); and DR5 (International application publication number WO 98/41629)); multimeric forms of soluble TRAIL receptor polypeptides; and TRAIL receptor antibodies that bind the TRAIL receptor without transducing the biological signal that results in apoptosis, anti-TRAIL antibodies that block binding of TRAIL to one or more TRAIL receptors, and muteins of TRAIL that bind TRAIL receptors but do not transduce the biological signal that results in apoptosis. Preferably, the antibodies employed according to this method are monoclonal antibodies.

[0356] TR16 polypeptides or polynucleotides encoding TR16 of the invention may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

[0357] Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogly of Fallot, ventricular heart septal defects, and conditions characterized by clotting of small blood vessels.

[0358] Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy,

post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

[0359] Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

[0360] Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

[0361] Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

[0362] Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

[0363] Cardiovascular diseases also include vascular diseases such as aneurysms, angiodyplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases,

arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, thrombotic microangiopathies (e.g., thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS)), and venous insufficiency.

[0364] Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

[0365] Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

[0366] Cerebrovascular disorders include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

[0367] Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

[0368] Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and

Wegener's granulomatosis.

[0369] The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

[0370] The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of the TR16 polynucleotides and/or polypeptides of the invention (including TR16 agonists and/or antagonists). Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides of the invention include, but are not limited to those malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)).

[0371] Additionally, ocular disorders associated with neovascularization which can be treated with the TR16 polynucleotides and polypeptides of the present invention (including TR16 agonists and TR16 antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization,

as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

[0372] Additionally, disorders which can be treated with the TR16 polynucleotides and polypeptides of the present invention (including TR16 agonists and TR16 antagonists) include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

[0373] The polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof, can also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

[0374] The polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof, may also be employed for the expansion of immature hematopoietic progenitor cells, for example, granulocytes, macrophages or monocytes (e.g., C-kit⁺, Sca-1⁺), by temporarily preventing their differentiation. These bone marrow cells may be cultured *in vitro*. Thus, TR16 may be useful as a modulator of hematopoietic stem cells *in vitro* for the purpose of bone marrow transplantation and/or gene therapy. Since stem cells are rare and are most useful for introducing genes into for gene therapy, TR16 can be used to isolate enriched populations of stem cells. Stem cells can be enriched by culturing cells in the presence of cytotoxins, such as 5-Fu, which kills rapidly dividing cells, where as the stem cells will be protected by TR16. These stem cells can be returned to a bone marrow transplant patient or can then be used for transfection of the desired gene for gene therapy. In addition, TR16 can be injected into animals which results in the release of stem cells from the bone marrow of the animal into the peripheral blood. These stem cells can be isolated for the purpose of autologous bone marrow transplantation or manipulation for gene therapy. After the patient has finished chemotherapy or radiation treatment, the isolated stem cells can be returned to the patient.

[0375] In a specific embodiment, polynucleotides and/or polypeptides of the invention

and/or agonists and/or antagonists thereof may be used to increase the concentration of blood cells in individuals in need of such increase (i.e., in hematopoietic therapy). Conditions that may be ameliorated by administering the compositions of the invention include, but are not limited to, neutropenia, anemia, and thrombocytopenia.

[0376] In a specific embodiment, the polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) are used in erythropoietin therapy, which is directed toward supplementing the oxygen carrying capacity of blood. Polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) may be used to treat or prevent diseases or conditions in patients generally requiring blood transfusions, such as, for example, trauma victims, surgical patients, dialysis patients, and patients with a variety of blood composition-affecting disorders, such as, for example, hemophilia, cystic fibrosis, pregnancy, menstrual disorders, early anemia of prematurity, spinal cord injury, aging, various neoplastic disease states, and the like. Examples of patient conditions that require supplementation of the oxygen carrying capacity of blood and which are within the scope of this invention, include, but are not limited to: treatment of blood disorders characterized by low or defective red blood cell production, anemia associated with chronic renal failure, stimulation of reticulocyte response, development of ferrokinetic effects (such as plasma iron turnover effects and marrow transit time effects), erythrocyte mass changes, stimulation of hemoglobin C synthesis, and increasing levels of hematocrit in vertebrates. The invention also provides for treatment to enhance the oxygen-carrying capacity of an individual, such as for example, an individual encountering hypoxic environmental conditions.

[0377] TR16 polynucleotides, polypeptides and/or agonists or antagonists may also be employed to regulate hematopoiesis, by regulating the activation and differentiation of various hematopoietic progenitor cells, for example, to release mature leukocytes from the bone marrow following chemotherapy, i.e., in stem cell mobilization. TR16 polynucleotides, polypeptides and/or agonists or antagonists may also be employed to treat sepsis.

[0378] TR16 polynucleotides, polypeptides and/or agonists or antagonists may also be employed to inhibit T-cell proliferation by the inhibition of IL-2 biosynthesis for the treatment of T-cell mediated auto-immune diseases and lymphocytic leukemias (including, for example, chronic lymphocytic leukemia (CLL) and large granular

lymphocytic (LGL) leukemia).

[0379] TR16 polynucleotides, polypeptides and/or agonists or antagonists may also be employed to stimulate wound healing, both via the recruitment of debris clearing and connective tissue promoting inflammatory cells. In this same manner, TR16 polynucleotides, polypeptides and/or agonists or antagonists may also be employed to treat other fibrotic disorders, including liver cirrhosis, osteoarthritis and pulmonary fibrosis.

[0380] TR16 polynucleotides, polypeptides and/or agonists or antagonists may also be employed to enhance host defenses against resistant chronic and acute infections, for example, myobacterial infections via the attraction and activation of microbicidal leukocytes.

[0381] TR16 polynucleotides, polypeptides and/or agonists or antagonists also increases the presence of eosinophils which have the distinctive function of killing the larvae of parasites that invade tissues, as in schistosomiasis, trichinosis and ascariasis.

[0382] TR16 polynucleotides or polypeptides, or agonists of TR16, can be used in the treatment of infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, TR16 polynucleotides or polypeptides, or agonists or antagonists of TR16, may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

[0383] Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated by TR16 polynucleotides or polypeptides, or agonists or antagonists of TR16. Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses

falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. TR16 polynucleotides or polypeptides, or agonists or antagonists of TR16, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, TR16 polynucleotides, polypeptides, or agonists are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment TR16 polynucleotides, polypeptides, or agonists are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment, TR16 polynucleotides, polypeptides, or agonists are used to treat AIDS.

[0384] Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated by TR16 polynucleotides or polypeptides, or agonists or antagonists of TR16, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Meningococcal), Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but

not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. TR16 polynucleotides or polypeptides, or agonists or antagonists of TR16, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, TR16 polynucleotides, polypeptides, or agonists thereof are used to treat: tetanus, Diphtheria, botulism, and/or meningitis type B.

[0385] Moreover, parasitic agents causing disease or symptoms that can be treated by TR16 polynucleotides or polypeptides, or agonists or antagonists of TR16, include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. TR16 polynucleotides or polypeptides, or agonists or antagonists of TR16, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, TR16 polynucleotides, polypeptides, or agonists or antagonists thereof are used to treat malaria.

[0386] In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing TR16 polypeptides or anti-TR16 antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells, such as, for example, B cells expressing TR16, or monocytes expressing the cell surface bound form of a TNF ligand that binds TR16. TR16 polypeptides of the invention, TNF ligands that bind TR16,

or anti-TR16 antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

[0387] In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., TR16 polypeptides or anti-TR16 antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[0388] In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., TR16 polypeptides or anti-TR16 antibodies) in association with toxins or cytotoxic prodrugs.

[0389] In a specific embodiment, the invention provides a method for the specific destruction of cells of B cell lineage (e.g., B cell related leukemias or lymphomas) by administering anti-TR16 antibodies or TNF ligands that bind TR16, in association with toxins or cytotoxic prodrugs.

[0390] In another specific embodiment, the invention provides a method for the specific destruction of cells of monocytic lineage (e.g., monocytic leukemias or lymphomas) by administering TR16 polypeptides of the invention (e.g., soluble TR16 polypeptides) in association with toxins or cytotoxic prodrugs.

[0391] By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and

cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

[0392] An additional condition, disease or symptom that can be treated by TR16 polynucleotides or polypeptides, or agonists or antagonist of TR16, is osteomyelitis.

[0393] Preferably, treatment using TR16 polynucleotides or polypeptides, or agonists or antagonists of TR16, could either be by administering an effective amount of TR16 polynucleotide or polypeptide to the patient, or by removing cells from the patient, supplying the cells with TR16 polynucleotide, and returning the engineered cells to the patient (ex vivo therapy). Moreover, as further discussed herein, the TR16 polypeptide or polynucleotide can be used as an adjuvant in a vaccine to raise an immune response against infectious disease.

[0394] Additional preferred embodiments of the invention include, but are not limited to, the use of TR16 polypeptides, TR16 polynucleotides, TR16 antibodies and functional agonists thereof, in the following applications:

[0395] Administration to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response.

[0396] Administration to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741).

[0397] A vaccine adjuvant that enhances immune responsiveness to specific antigen.

In a specific embodiment, the vaccine adjuvant is a TR16 polypeptide described herein. In another specific embodiment, the vaccine adjuvant is a TR16 polynucleotide described herein (i.e., the TR16 polynucleotide is a genetic vaccine adjuvant). As discussed herein, TR16 polynucleotides may be administered using techniques known in the art, including but not limited to, liposomal delivery, recombinant vector delivery, injection of naked DNA, and gene gun delivery.

[0398] An adjuvant to enhance tumor-specific immune responses.

[0399] An adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus, Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles, Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex, and yellow fever. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to the HIV gp120 antigen.

[0400] An adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, *Borrelia*

burgdorferi, and *Plasmodium* (malaria).

[0401] An adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to *Plasmodium* (malaria).

[0402] As a stimulator of B cell responsiveness to pathogens.

[0403] As an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

[0404] As an agent to induce higher affinity antibodies.

[0405] As an agent to increase serum immunoglobulin concentrations.

[0406] As an agent to accelerate recovery of immunocompromised individuals.

[0407] As an agent to boost immunoresponsiveness among aged populations.

[0408] As an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

[0409] As an agent to boost immunoresponsiveness among B cell immunodeficient individuals. B cell immunodeficiencies that may be ameliorated or treated by administering the TR16 polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified

hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVI) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic aplasia-aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

[0410] In a specific embodiment, TR16 polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat or ameliorate selective IgA deficiency.

[0411] In another specific embodiment, TR16 polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat or ameliorate ataxia-telangiectasia.

[0412] In another specific embodiment, TR16 polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat or ameliorate common variable immunodeficiency.

[0413] In another specific embodiment, TR16 polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat or ameliorate X-linked agammaglobulinemia.

[0414] In another specific embodiment, TR16 polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat or ameliorate severe combined immunodeficiency (SCID).

[0415] In another specific embodiment, TR16 polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat or ameliorate Wiskott-Aldrich syndrome.

[0416] In another specific embodiment, TR16 polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat or ameliorate severe combined immunodeficiency (SCID).

[0417] In another specific embodiment, TR16 polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat or ameliorate X-linked Ig deficiency with hyper IgM.

[0418] As an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the TR16 polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

[0419] As an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the TR16 polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, recovery from surgery.

[0420] As a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, TR16 polypeptides (in soluble, membrane-bound or transmembrane forms) or polynucleotides enhance antigen presentation or antagonize antigen presentation in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonism of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system.

[0421] As a mediator of mucosal immune responses.

[0422] As an agent to direct an individuals immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

[0423] As a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

[0424] As a monocyte cell specific binding protein to which specific activators or inhibitors of cell growth may be attached. The result would be to focus the activity of such activators or inhibitors onto normal, diseased, or neoplastic B cell populations.

- [0425] As a means of detecting B-lineage cells.
- [0426] As a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency.
- [0427] As a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect.
- [0428] As a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence such as observed among SCID patients.
- [0429] As an antigen for the generation of antibodies to inhibit or enhance TR16 mediated responses.
- [0430] As a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leishmaniasis.
- [0431] As pretreatment of bone marrow samples prior to transplant. Such treatment would increase B cell representation and thus accelerate recover.
- [0432] As a means of regulating secreted cytokines that are elicited by TR16.
- [0433] TR16 polypeptides or polynucleotides of the invention, or agonists may be used to modulate IgE concentrations in vitro or in vivo.
- [0434] Additionally, TR16 polypeptides or polynucleotides of the invention, or agonists thereof, may be used to treat or prevent IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema.
- [0435] All of the above described applications as they may apply to veterinary medicine.
- [0436] Antagonists of TR16 include binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes, soluble forms of TR16, or TNF-ligands that bind TR16. These would be expected to reverse many of the activities of the ligand described above as well as find clinical or practical application as:
- [0437] A means of blocking various aspects of immune responses to foreign agents or self. Examples include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and pathogens.
- [0438] A therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus and MS.

- [0439] An inhibitor of graft versus host disease or transplant rejection.
- [0440] A therapy for B cell malignancies such as ALL, Hodgkin's disease, non-Hodgkin's lymphoma, Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases.
- [0441] A therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonal gammopathies, and plasmacytomas.
- [0442] A therapy for decreasing cellular proliferation of Large B-cell Lymphomas.
- [0443] A means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.
- [0444] As a B cell specific binding protein to which specific activators or inhibitors of cell growth may be attached. The result would be to focus the activity of such activators or inhibitors onto normal, diseased, or neoplastic B cell populations.
- [0445] As part of a B cell selection device the function of which is to isolate B cells from a heterogeneous mixture of cell types. Anti-TR16 antibody or TNF ligands that bind TR16 could be coupled to a solid support to which B cells would then specifically bind. Unbound cells would be washed out and the bound cells subsequently eluted. This technique would allow purging of tumor cells from, for example, bone marrow or peripheral blood prior to transplant.
- [0446] An immunosuppressive agent(s).
- [0447] TR16 polypeptides or polynucleotides of the invention, or antagonists may be used to modulate IgE concentrations in vitro or in vivo.
- [0448] In another embodiment, administration of TR16 polypeptides or polynucleotides of the invention, or antagonists thereof, may be used to treat or prevent IgE-mediated allergic reactions including, but not limited to, asthma, rhinitis, and eczema.
- [0449] An inhibitor of signaling pathways involving ERK1, COX2 and Cyclin D2 which have been associated with TR16 induced B cell activation.
- [0450] The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a

mammal. In most preferred embodiments, the host is a human.

[0451] The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described above.

[0452] The antagonists may be employed for instance to inhibit the chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain auto-immune and chronic inflammatory and infective diseases. Examples of auto-immune diseases include multiple sclerosis, and insulin-dependent diabetes. The antagonists may also be employed to treat infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by preventing the recruitment and activation of mononuclear phagocytes. They may also be employed to treat idiopathic hyper-eosinophilic syndrome by preventing eosinophil production and migration. Endotoxic shock may also be treated by the antagonists by preventing the migration of macrophages and their production of the TR16 polypeptides of the present invention. The antagonists may also be employed for treating atherosclerosis, by preventing monocyte infiltration in the artery wall. The antagonists may also be employed to treat histamine-mediated allergic reactions and immunological disorders including late phase allergic reactions, chronic urticaria, and atopic dermatitis by inhibiting chemokine-induced mast cell and basophil degranulation and release of histamine. IgE-mediated allergic reactions such as allergic asthma, rhinitis, and eczema may also be treated. The antagonists may also be employed to treat chronic and acute inflammation by preventing the attraction of monocytes to a wound area. They may also be employed to regulate normal pulmonary macrophage populations, since chronic and acute inflammatory pulmonary diseases are associated with sequestration of mononuclear phagocytes in the lung. Antagonists may also be employed to treat rheumatoid arthritis by preventing the attraction of monocytes into synovial fluid in the joints of patients. Monocyte influx and activation plays a significant role in the pathogenesis of both degenerative and inflammatory arthropathies. The antagonists may be employed to interfere with the deleterious cascades attributed primarily to IL-1 and TNF, which prevents the biosynthesis of other inflammatory cytokines. In this way, the antagonists may be employed to prevent inflammation. The antagonists may also be employed to inhibit prostaglandin-independent fever induced by TR16. The antagonists may also be

employed to treat cases of bone marrow failure, for example, aplastic anemia and myelodysplastic syndrome. The antagonists may also be employed to treat asthma and allergy by preventing eosinophil accumulation in the lung. The antagonists may also be employed to treat subepithelial basement membrane fibrosis which is a prominent feature of the asthmatic lung. The antagonists may also be employed to treat lymphomas (e.g., one or more of the extensive, but not limiting, list of lymphomas provided herein).

[0453] Antibodies against TR16 may be employed to bind to and inhibit TR16 activity to treat ARDS, by preventing infiltration of neutrophils into the lung after injury. The antagonists and antagonists of the instant may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described hereinafter.

[0454] TR16 polynucleotides, polypeptides, and/or agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

[0455] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in the diagnosis and treatment or prevention of a wide range of diseases and/or conditions. Such diseases and conditions include, but are not limited to, cancer (e.g., immune cell related cancers, breast cancer, prostate cancer, ovarian cancer, follicular lymphoma, cancer associated with mutation or alteration of p53, brain tumor, bladder cancer, uterocervical cancer, colon cancer, colorectal cancer, non-small cell carcinoma of the lung, small cell carcinoma of the lung, stomach cancer, etc.), lymphoproliferative disorders (e.g., lymphadenopathy), microbial (e.g., viral, bacterial, etc.) infection (e.g., HIV-1 infection, HIV-2 infection, herpesvirus infection (including, but not limited to, HSV-1, HSV-2, CMV, VZV, HHV-6, HHV-7, EBV), adenovirus infection, poxvirus infection, human papilloma virus infection, hepatitis infection (e.g., HAV, HBV, HCV, etc.), *Helicobacter pylori* infection, invasive Staphylococci, etc.), parasitic infection, nephritis, bone disease (e.g., osteoporosis), atherosclerosis, pain, cardiovascular disorders (e.g., neovascularization, hypovascularization or reduced circulation (e.g., ischemic disease (e.g., myocardial infarction, stroke, etc.))), AIDS, allergy, inflammation, neurodegenerative disease (e.g., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, pigmentary retinitis, cerebellar degeneration, etc.), graft rejection (acute and chronic), graft vs. host disease, diseases due to osteomyelodysplasia (e.g., aplastic anemia, etc.), joint tissue destruction in rheumatism,

liver disease (e.g., acute and chronic hepatitis, liver injury, and cirrhosis), autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis.

[0456] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in promoting angiogenesis, regulating hematopoiesis and wound healing (e.g., wounds, burns, and bone fractures).

[0457] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are also useful as an adjuvant to enhance immune responsiveness to specific antigen, anti-viral immune responses.

[0458] More generally, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in regulating (i.e., elevating or reducing) immune response. For example, polynucleotides and/or polypeptides of the invention may be useful in preparation or recovery from surgery, trauma, radiation therapy, chemotherapy, and transplantation, or may be used to boost immune response and/or recovery in the elderly and immunocompromised individuals. Alternatively, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful as immunosuppressive agents, for example in the treatment or prevention of autoimmune disorders. In specific embodiments, polynucleotides and/or polypeptides of the invention are used to treat or prevent chronic inflammatory, allergic or autoimmune conditions, such as those described herein or are otherwise known in the art.

[0459] In one aspect, the present invention is directed to a method for enhancing TR16 mediated signaling by a TNF-family ligand, which involves administering to a cell which expresses the TR16 polypeptide an effective amount of TR16 ligand, analog or an agonist capable of increasing TR16 mediated signaling. Preferably, TR16 mediated signaling is increased to treat a disease wherein increased apoptosis, decreased cytokine and adhesion molecule expression, or decreased cell proliferation is exhibited. An agonist can include soluble forms of TR16 and monoclonal antibodies directed against the TR16 polypeptide.

[0460] In a further aspect, the present invention is directed to a method for inhibiting

TR16 mediated signaling induced by a TNF-family ligand, which involves administering to a cell which expresses the TR16 polypeptide an effective amount of an antagonist capable of decreasing TR16 mediated signaling. Preferably, TR16 mediated signaling is decreased to treat a disease wherein decreased apoptosis or NF-kB expression, or increased cell proliferation, is exhibited. An antagonist can include soluble forms of TR16 and monoclonal antibodies directed against the TR16 polypeptide.

[0461] By "agonist" is intended naturally occurring and synthetic compounds capable of enhancing or potentiating TR16 mediated signaling. By "antagonist" is intended naturally occurring and synthetic compounds capable of inhibiting apoptosis. Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit Tr16 mediated signaling can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below.

[0462] One such screening procedure involves the use of melanophores which are transfected to express the receptor of the present invention. Such a screening technique is described in PCT WO 92/01810. Such an assay may be employed, for example, for screening for a compound which inhibits (or enhances) activation of the receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptor with both a TNF-family ligand and the candidate antagonist (or agonist). Inhibition or enhancement of the signal generated by the ligand indicates that the compound is an antagonist or agonist of the ligand/receptor signaling pathway.

[0463] Other screening techniques include the use of cells which express the receptor (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation. For example, compounds may be contacted with a cell which expresses the receptor polypeptide of the present invention and a second messenger response, e.g., signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the receptor.

[0464] Another such screening technique involves introducing RNA encoding the receptor into *Xenopus* oocytes to transiently express the receptor. The receptor oocytes may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

[0465] Another screening technique well known in the art involves expressing in cells

a construct wherein the receptor is linked to a phospholipase C or D. Exemplary cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase signal.

[0466] Another method involves screening for compounds which inhibit activation of the receptor polypeptide of the present invention antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the receptor such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

[0467] Further screening assays for agonists and antagonists of the present invention are described in L.A. Tartaglia and D.V. Goeddel, *J. Biol. Chem.* 267:4304-4307(1992).

[0468] Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand. The method involves contacting cells which express the TR16 polypeptide with a candidate compound and a TNF-family ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or a TNF-family ligand (e.g., determining or estimating an increase or decrease in B and/or T cell proliferation or tritiated thymidine labeling). By the invention, a cell expressing the TR16 polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand.

[0469] Agonists according to the present invention include naturally occurring and

synthetic compounds such as, for example, the CD40 ligand, neutral amino acids, zinc, estrogen, androgens, viral genes (such as Adenovirus *ElB*, Baculovirus *p35* and *IAP*, Cowpox virus *crmA*, Epstein-Barr virus *BHRF1*, *LMP-1*, African swine fever virus *LMW5-HL*, and Herpesvirus *yl 34.5*), calpain inhibitors, cysteine protease inhibitors, and tumor promoters (such as PMA, Phenobarbital, and Hexachlorocyclohexanes).

[0470] Antagonist according to the present invention include naturally occurring and synthetic compounds such as, for example, TNF family ligand peptide fragments, transforming growth factor, neurotransmitters (such as glutamate, dopamine, *N*-methyl-D-aspartate), tumor suppressors (p53), cytolytic T cells and antimetabolites. Preferred agonists include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and -amyloid peptide. (*Science* 267:1457-1458 (1995)). Further preferred agonists include Tr16 polypeptides of the invention, polyclonal and monoclonal antibodies raised against the TR16 polypeptide, or a fragment thereof. Such agonist antibodies raised against a TNF-family receptor are disclosed in L.A. Tartaglia *et al.*, *Proc. Natl. Acad. Sci. USA* 88:9292-9296 (1991); and L.A.Tartaglia and D.V.Goeddel, *J. Biol. Chem.* 267:4304- 4307(1992). See, also, PCT Application WO 94/09137.

[0471] Other potential antagonists according to the invention include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, *J. Neurochem.* 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee *et al.*, *Nucleic Acids Research* 6:3073 (1979); Cooney *et al.*, *Science* 241:456 (1988); and Dervan *et al.*, *Science* 251:1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

[0472] In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in TR16 (Figures 1A-E; SEQ ID NO:1), or the complementary strand thereof, and/or to nucleotide sequences contained in the deposited clone ATCC Deposit No. PTA-506. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, Okano H. *et al.*, *J. Neurochem.*

56:560 (1991), and Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, *Neurochem.* 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee *et al.*, *Nucleic Acids Research* 6:3073 (1979); Cooney *et al.*, *Science* 241:456 (1988); and Dervan *et al.*, *Science* 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

[0473] For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into receptor polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the receptor.

[0474] In one embodiment, the TR16 antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the TR16 antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding TR16, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature* 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, *Cell* 22:787-797 (1980), the herpes thymidine promoter (Wagner *et al.*, *Proc. Natl. Acad. Sci.*

U.S.A. 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, *et al.*, *Nature* 296:39-42 (1982)), etc.

[0475] The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a TR16 gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded TR16 antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a TR16 RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0476] Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature* 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of the TR16 shown in Figures 1A-E could be used in an antisense approach to inhibit translation of endogenous TR16 mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. While antisense nucleotides complementary to the TR16 coding region sequence may be used, those complementary to the transcribed untranslated region are most preferred. Whether designed to hybridize to the 5'-, 3'- or coding region of TR16 mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

[0477] The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556 (1989); Lemaitre *et al.*, *Proc. Natl. Acad. Sci.* 84:648-652 (1987); PCT Publication No. WO88/09810) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134), hybridization-triggered cleavage agents. (See, e.g., Krol *et al.*, *BioTechniques* 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, *Pharm. Res.* 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0478] The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5_-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

[0479] The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

[0480] In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a

phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

[0481] In yet another embodiment, the antisense oligonucleotide is an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier *et al.*, *Nucl. Acids Res.* 15:6625-6641 (1987)). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue *et al.*, *Nucl. Acids Res.* 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue *et al.*, *FEBS Lett.* 215:327-330 (1987)).

[0482] Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein *et al.* (*Nucl. Acids Res.* 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 85:7448-7451 (1988)), etc.

[0483] Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364; Sarver *et al.*, *Science* 247:1222-1225 (1990)). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy TR16 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, *Nature* 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of TR16 (Figures 1A-E (SEQ ID NO:1)). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the TR16 mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

[0484] As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.) and should be

delivered to cells which express TR16 *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous TR16 messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

[0485] Endogenous gene expression can also be reduced by inactivating or "knocking out" the TR16 gene and/or its promoter using targeted homologous recombination. (E.g., see Smithies *et al.*, *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson *et al.*, *Cell* 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art. The contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety.

[0486] The techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of TR16 thereby effectively generating agonists and antagonists of TR16. See generally, International Publication No. WO 99/29902, U.S. Patent Nos.

5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten *et al.*, *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, *Trends Biotechnol.* 16(2):76-82 (1998); Hansson *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo and Blasco, *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of TR16 polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired TR16 molecule by homologous, or site-specific, recombination. In another embodiment, TR16 polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of TR16 may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are include, but are not limited to, TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), TRAIL, AIM-II (International Publication No. WO 97/34911), APRIL (*J. Exp. Med.* 188(6):1185-1190 (1998)), endokine-alpha (International Publication No. WO 98/07880), Neutrokin-alpha (International Publication No. WO98/18921), OPG, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153. In further preferred embodiments, the heterologous molecules are any member of the TNF family.

[0487] In other embodiments, antagonists according to the present invention include soluble forms of TR16 (e.g., fragments of the TR16 shown in Figures 1A-E (SEQ ID NO:2) that include one or more of the cysteine rich domains from the extracellular region of the full length receptor). Such soluble forms of the TR16, which may be naturally

occurring or synthetic, antagonize TR16 mediated signaling by competing with the cell surface bound forms of the receptor for binding to TNF-family ligands. Antagonists of the present invention also include antibodies specific for TNF-family ligands and TR16-Fc fusion proteins.

[0488] By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and inducing and/or blocking the ligand/receptor signaling pathway. Members of the TNF ligand family include, but are not limited to, TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), TRAIL, AIM-II (International Publication No. WO 97/34911), APRIL (*J. Exp. Med.* 188(6):1185-1190 (1998)), endokine-alpha (International Publication No. WO 98/07880), Neutrokin alpha (International Publication No. WO98/18921), OPG, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

[0489] TNF- α has been shown to protect mice from infection with herpes simplex virus type 1 (HSV-1). Rossol-Voth et al., *J. Gen. Virol.* 72:143-147 (1991). The mechanism of the protective effect of TNF- α is unknown but appears to involve neither interferons nor NK cell killing. One member of the family has been shown to mediate HSV-1 entry into cells. Montgomery et al., *Eur. Cytokine Newt.* 7:159 (1996). Further, antibodies specific for the extracellular domain of this block HSV-1 entry into cells. Thus, TR16 antagonists of the present invention include both TR16 amino acid sequences and antibodies capable of preventing mediated viral entry into cells. Such sequences and antibodies can function by either competing with cell surface localized for binding to virus or by directly blocking binding of virus to cell surface receptors.

[0490] Antibodies according to the present invention may be prepared by any of a

variety of methods using TR16 antigens (e.g., immunogens) of the present invention. As indicated, such TR16 antigens include the full length TR16 polypeptide (which may or may not include the leader sequence) and TR16 polypeptide fragments such as the extracellular domain, the cysteine rich domain, one or more of the TR16 cysteine-rich domains, the transmembrane domain, and the intracellular domain, or any combination thereof.

[0491] Polyclonal and monoclonal antibody agonists or antagonists according to the present invention can be raised according to the methods disclosed herein and and/or known in the art, such as, for example, those methods described in Tartaglia and Goeddel, *J. Biol. Chem.* 267(7):4304-4307(1992); Tartaglia *et al.*, *Cell* 73:213-216 (1993), and PCT Application WO 94/09137 (the contents of each of these three publications are herein incorporated by reference in their entireties), and are preferably specific to TR16 polypeptides of the invention having the amino acid sequence of SEQ ID NO:2.

[0492] Antagonists according to the present invention include soluble forms of TR16, i.e., TR16 fragments that include one or more of the cysteine rich domains from the extracellular region of the full length receptor. Such soluble forms of the receptor, which may be naturally occurring or synthetic, antagonize TR16 mediated signaling by competing with the cell surface TR16 for binding to TNF-family ligands. Thus, soluble forms of the receptor that include one or more of the cysteine-rich motifs of TR16 are novel cytokines capable of inhibiting TR16 mediated signaling induced by TNF-family ligands. These soluble forms are preferably expressed as dimers or trimers, since these have been shown to be superior to monomeric forms of soluble receptor as antagonists, e.g., IgGFc-TNF receptor family fusions. Other such cytokines are known in the art and include Fas B (a soluble form of the mouse Fas receptor) that acts physiologically to limit apoptosis induced by Fas ligand (D.P. Hughes and I.N. Crispe, *J. Exp. Med.* 182:1395-1401 (1995)).

[0493] Proteins and other compounds which bind the TR16 domains are also candidate agonists and antagonists according to the present invention. Such binding compounds can be "captured" using the yeast two-hybrid system (Fields and Song, *Nature* 340:245-246 (1989)). A modified version of the yeast two-hybrid system has been described by Roger Brent and his colleagues (J. Gyuris, *Cell* 75:791-803 (1993); A.S. Zervos *et al.*, *Cell* 72:223-232 (1993)). Preferably, the yeast two-hybrid system is used

according to the present invention to capture compounds which bind to either one or more of the TR16 extracellular rich motifs or to the TR16 intracellular domain. Such compounds are good candidate agonists and antagonists of the present invention.

MODES OF ADMINISTRATION

[0494] The agonist or antagonists described herein, including but not limited to antibodies, peptides, and small organic molecules, can be administered *in vitro*, *ex vivo*, or *in vivo* to cells which express the receptor of the present invention. By administration of an "effective amount" of an agonist or antagonist is intended an amount of the compound that is sufficient to enhance or inhibit a cellular response to a TNF-family. One of ordinary skill will appreciate that effective amounts of an agonist or antagonist can be determined empirically and may be employed in pure form or in pharmaceutically acceptable salt, ester or prodrug form. The agonist or antagonist may be administered in compositions in combination with one or more pharmaceutically acceptable excipients.

[0495] It will be understood that, when administered to a human patient, the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon factors well known in the medical arts.

[0496] As a general proposition, the total pharmaceutically effective amount of TR16 polypeptide administered parenterally per dose will be in the range of about 1 ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the TR16 polypeptide is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

[0497] Dosage may also be arranged in a patient specific manner to provide a predetermined concentration of an agonist or antagonist in the blood, as determined by the RIA technique. Thus patient dosage may be adjusted to achieve regular on-going trough blood levels, as measured by RIA, on the order of from 50 to 1000 ng/ml, preferably 150

to 500 ng/ml.

[0498] Pharmaceutical compositions containing the TR16 polypeptide of the invention may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intracisternal, subcutaneous and intraarticular injection and infusion.

[0499] Pharmaceutical compositions of the present invention for parenteral injection can comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[0500] In addition to soluble TR16 polypeptides, TR16 polypeptides containing the transmembrane region can also be used when appropriately solubilized by including detergents, such as CHAPS or NP-40, with buffer.

[0501] TR16 compositions of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

[0502] Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., *Id.*) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

[0503] Sustained-release compositions also include liposomally entrapped

compositions of the invention (see generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing TR16 polypeptide may be prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. (USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal TR16 polypeptide therapy.

[0504] In yet an additional embodiment, the compositions of the invention are delivered by way of a pump (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)).

[0505] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990), which is hereby incorporated herein by reference in its entirety).

[0506] The compositions of the invention may be administered alone or in combination with other adjuvants. Adjuvants that may be administered with the compositions of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, compositions of the invention are administered in combination with alum. In another specific embodiment, compositions of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the compositions of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the compositions of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, *Haemophilus influenzae* B, whooping cough, pneumonia, influenza, Lyme Disease, rotavirus, cholera, yellow fever, Japanese encephalitis,

poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0507] The compositions of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the compositions of the invention, include but are not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, antivirals, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines, chemokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0508] In one embodiment, the compositions of the invention are administered in combination with other members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the compositions of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), TRAIL, AIM-II (International Publication No. WO 97/34911), APRIL (*J. Exp. Med.* 188(6):1185-1190 (1998)), endokine-alpha (International Publication No. WO 98/07880), Neutrokine-alpha (International Application Publication No. WO 98/18921), OPG, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No.

WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

[0509] In certain embodiments, compositions of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with compositions of the invention to treat AIDS and/or to prevent or treat HIV infection.

[0510] In other embodiments, compositions of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the compositions of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, compositions of the invention are used in any combination with

TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, compositions of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, compositions of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, compositions of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, compositions of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, compositions of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICLOVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, compositions of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, compositions of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

[0511] In a further embodiment, the compositions of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the compositions of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

[0512] In a further embodiment, the compositions of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the compositions of the invention include, but are not limited to, amoxicillin, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, clindamycin,

chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamethoxazole, and vancomycin.

[0513] Conventional nonspecific immunosuppressive agents, that may be administered in combination with the compositions of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

[0514] Additional immunosuppressants preparations that may be administered with the compositions of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

[0515] In an additional embodiment, compositions of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the compositions of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, compositions of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

[0516] In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the compositions of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide,

orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

[0517] In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the compositions of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., methoxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephallen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

[0518] In a specific embodiment, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, compositions of the invention are administered in combination with Rituximab. In a further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination one or more of the components of CHOP.

[0519] In an additional embodiment, the compositions of the invention are administered in combination with cytokines. Cytokines that may be administered with the compositions of the invention include, but are not limited to, GM-CSF, G-CSF, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In one embodiment, the compositions of the invention are administered in combination with one or more chemokines. In specific embodiments, the compositions of the invention are administered in combination with an α (CxC) chemokine selected from the group consisting of gamma-interferon inducible protein-10 (γ IP-10), interleukin-8 (IL-8), platelet factor-4 (PF4), neutrophil activating protein (NAP-2), GRO- α , GRO- β , GRO-

γ, neutrophil-activating peptide (ENA-78), granulocyte chemoattractant protein-2 (GCP-2), and stromal cell-derived factor-1 (SDF-1, or pre-B cell stimulatory factor (PBSF)); and/or a β(CC) chemokine selected from the group consisting of: RANTES (regulated on activation, normal T expressed and secreted), macrophage inflammatory protein-1 alpha (MIP-1α), macrophage inflammatory protein-1 beta (MIP-1β), monocyte chemotactic protein-1 (MCP-1), monocyte chemotactic protein-2 (MCP-2), monocyte chemotactic protein-3 (MCP-3), monocyte chemotactic protein-4 (MCP-4) macrophage inflammatory protein-1 gamma (MIP-1γ), macrophage inflammatory protein-3 alpha (MIP-3α), macrophage inflammatory protein-3 beta (MIP-3β), macrophage inflammatory protein-4 (MIP-4/DC-CK-1/PARC), eotaxin, Exodus, and I-309; and/or the γ(C) chemokine, lymphotactin.

[0520] In an additional embodiment, the compositions of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the compositions of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

[0521] The invention also encompasses combining the polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) with other proposed or conventional hematopoietic therapies. Thus, for example, the polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) can be combined with compounds that singly exhibit erythropoietic stimulatory effects, such as erythropoietin, testosterone, progenitor cell stimulators, insulin-like growth factor, prostaglandins, serotonin, cyclic AMP, prolactin, and triiodothyronine. Also encompassed are combinations of the compositions of the invention with compounds generally used to treat aplastic anemia, such as, for example, methenolene, stanozolol, and nandrolone; to treat iron-deficiency anemia, such as, for example, iron preparations; to treat malignant anemia, such as, for example, vitamin B₁₂ and/or folic acid; and to treat hemolytic anemia, such as, for example, adrenocortical steroids, e.g., corticoids. See e.g., Resegotti et al., *Panminerva Medica*, 23:243-248 (1981); Kurtz, *FEBS Letters*, 14a:105-108 (1982); McGonigle et al., *Kidney Int.*, 25:437-444 (1984); and Pavlovic-Kantera, *Expt. Hematol.*, 8(supp. 8) 283-291 (1980), the contents of each of which are hereby incorporated by reference in their entireties.

antibody or peptide of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0526] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[0527] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0528] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes,

such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0529] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, 1990, *Science* 249:1527-1533; Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*).

[0530] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:201; Buchwald et al., 1980, *Surgery* 88:507; Saudek et al., 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., 1983, *Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, *J. Neurosurg.* 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

[0531] Other controlled release systems are discussed in the review by Langer (1990, *Science* 249:1527-1533).

[0532] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:1864-1868), etc. Alternatively, a nucleic acid can be

introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0533] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0534] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration

are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0535] The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0536] The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0537] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of

antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

[0538] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

CHROMOSOME ASSAYS

[0539] The nucleic acid molecules of the present invention are also valuable for chromosome identification. TR16 has been mapped to chromosome 7q21. Accordingly, TR16 polynucleotides related to this invention are useful as markers in linkage analysis for chromosome 7q21; an important first step in correlating those sequences with genes associated with disease. Chromosomal rearrangements in 7q21 have been implicated in lymphomas (see, e.g., Schlegelberger et al., Cancer Genet Cytogenet 78:15-22 (1994); and Mateo et al., Am. J. Pathol., 154:1583-9 (1999); incorporated herein by reference).

[0540] In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a TR16 receptor gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA is then used for *in situ* chromosome mapping using well known techniques for this purpose.

[0541] In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes.

[0542] Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al.*, *Human Chromosomes: a Manual of Basic Techniques*,

Pergamon Press, New York (1988).

[0543] Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man*, available on line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

[0544] Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

[0545] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

EXAMPLES

EXAMPLE 1

EXPRESSION AND PURIFICATION OF THE TR16-SHORT RECEPTOR IN *E. COLI*

[0546] The bacterial expression vector pHE4 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pHE4 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide may be inserted in such a way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, in this example, the polypeptide coding sequence is inserted such that translation of the six His codons is prevented and, therefore, the polypeptide is produced with no 6 X His tag.

[0547] The DNA sequence encoding the desired portion of the TR16 protein lacking the hydrophobic leader sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the TR16 protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pHE4 vector are added to the 5' and 3' sequences, respectively.

[0548] For cloning the mature protein, the 5' primer has the sequence: 5'-GCAGCA CATATGGGGGGACCTGCCCTCCTCCTCCAGCCGCCCCGCTTC-3' (SEQ ID NO:36) containing the underlined NcoI restriction site followed by nucleotides complementary to the amino terminal coding sequence of the mature TR16 sequence in Figures 1A-E. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a desired portion of the complete protein shorter or longer than the mature form.

[0549] The 3' primer has the sequence: 5'-GCAGCAACTAGTTTAGTCAAC CGTTTCACAGGTTGCCAACTTTTTC-3' (SEQ ID NO:37) containing the underlined SpeI site followed by nucleotides complementary to the 3' end of the non-coding sequence in the TR16 DNA sequence in Figures 1A-E.

[0550] The amplified TR16 DNA fragments and the vector pHE4 are digested with Nco I and SpeI and the digested DNAs then ligated together. Insertion of the TR16 protein DNA into the restricted pHE4 vector places the TR16 protein coding region (including its associated stop codon) downstream from the IPTG-inducible promoter and in-frame with an initiating AUG. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

[0551] The ligation mixture is transformed into competent *E. coli* cells using standard procedures. Such procedures are described in Sambrook *et al.*, *Molecular Cloning: a Laboratory Manual*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing TR16 protein, is available commercially from Qiagen, Inc., *supra*.

[0552] Transformants are identified by their ability to grow on LB plates in the

presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR, and DNA sequencing.

[0553] Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100µg/ml) and kanamycin (25µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:100 to 1:250. The cells are grown to an optical density at 600nm ("OD600") of between 0.4 and 0.6. Isopropyl-B-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1mM to induce transcription from the *lac* repressor sensitive promoter, by inactivating the *lacI* repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

[0554] The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH8. The cell debris is removed by centrifugation, and the supernatant containing the TR16 is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the NI-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist, 1995, QIAGEN, Inc., *supra*). Briefly the supernatant is loaded onto the column in 6M guanidine-HCl, pH8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH8, then washed with 10 volumes of 6M guanidine-HCl pH6, and finally the TR16 is eluted with 6M guanidine-HCl, pH5.

[0555] The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50mM Na-acetate, pH 6 buffer plus 200mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500mM NaCl, 20% glycerol, 20mM Tris/HCl pH7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins can be eluted by the addition of 250mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50mM sodium acetate, pH6, buffer plus 200mM NaCl. The purified protein is stored at 4°C or frozen at -80°C.

EXAMPLE 1A

EXPRESSION AND PURIFICATION OF THE TR16-LONG RECEPTOR IN *E. COLI*

[0556] The bacterial expression vector pHE4 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pHE4 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide may be inserted in such a way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, in this example, the polypeptide coding sequence is inserted such that translation of the six His codons is prevented and, therefore, the polypeptide is produced with no 6 X His tag.

[0557] The DNA sequence encoding the desired portion of the TR16-long protein lacking the hydrophobic leader sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the TR16-long protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pHE4 vector are added to the 5' and 3' sequences, respectively.

[0558] For cloning the mature protein, the 5' primer has the sequence: 5'-GCAGCA CATATGGGGGACCTGCCCTCCTCCTCCAGCCGCCCGCTTC-3' (SEQ ID NO:36) containing the underlined NcoI restriction site followed by nucleotides complementary to the amino terminal coding sequence of the mature TR16-long sequence in Figures 4A-E (SEQ ID NO:4). One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a desired portion of the complete protein shorter or longer than the mature form.

[0559] The 3' primer has the sequence: 5'-GCAGCAGGTACCTCATATATTTGGGGATCTTGAGGTTTTCAG-3' (SEQ ID NO:38) containing the underlined Asp718 site followed by nucleotides complementary to the 3' end of the non-coding sequence in the TR16 DNA sequence in Figures 4A-E (SEQ ID NO:4).

[0560] The amplified TR16-long DNA fragments are digested with NcoI. To

overcome the fact that the coding region for TR16-long contains an internal Asp718 restriction site, the NcoI digested amplified fragments are then partially digested with Asp718 and fragments which are cleaved only at the 5' and 3' generated NcoI and Asp718 sites are selected for cloning. The vector pHE4 is digested with Nco I and Asp718 and the digested DNAs then ligated together. Insertion of the TR16-long protein DNA into the restricted pHE4 vector places the TR16-long protein coding region (including its associated stop codon) downstream from the IPTG-inducible promoter and in-frame with an initiating AUG. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

[0561] The ligation mixture is transformed into competent *E. coli* cells as above.

[0562] Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR, and DNA sequencing.

[0563] Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100µg/ml) and kanamycin (25µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:100 to 1:250. The cells are grown to an optical density at 600nm ("OD600") of between 0.4 and 0.6. Isopropyl-B-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1mM to induce transcription from the *lac* repressor sensitive promoter, by inactivating the *lacI* repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

[0564] The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH8. The cell debris is removed by centrifugation, and the supernatant containing the TR16 is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the NI-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist, 1995, QIAGEN, Inc., *supra*). Briefly the supernatant is loaded onto the column in 6M guanidine-HCl, pH8, the column is first washed with 10 volumes of 6M guanidine-HCl, pH8, then washed with 10 volumes of 6M guanidine-HCl pH6, and finally the TR16 is eluted with 6M guanidine-HCl, pH5.

[0565] The purified protein is then renatured as described above.

EXAMPLE 2

CLONING AND EXPRESSION OF TR16 IN A BACULOVIRUS EXPRESSION SYSTEM

[0566] In this illustrative example, the plasmid shuttle vector pA2 is used to insert the cloned DNA encoding the complete protein, including its naturally associated secretory signal (leader) sequence, into a baculovirus to express the mature TR16 protein, using standard methods as described in Summers *et al.*, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

[0567] Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow *et al.*, *Virology* 170:31-39 (1989).

[0568] The cDNA sequence encoding the mature TR16 receptor protein in the deposited clone, lacking the AUG initiation codon and the naturally associated leader sequence shown in Figures 1A-E (SEQ ID NO:2), is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene.

[0569] The 5' primer has the sequence 5'-GCAGCAAGATCTCCGCCATCATGCTG TTCCGCGCCCGGGGGCCGGTAC-3' (SEQ ID NO:39) containing the underlined BglII restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by M. Kozak, *J. Mol. Biol.* 196:947- 950 (1987), followed by bases of the sequence of the mature TR16 protein shown in Figures 1A-E, beginning with the indicated

N-terminus of the mature protein.

[0570] The 3' primer for TR16 has the sequence 5'-GCAGCAACTAGTTTAGTCAACC GTTTCACAGGTTGCCAACTTTTTC-3' (SEQ ID NO:37) containing the underlined SpeI restriction site followed by nucleotides complementary to the 3' non-coding sequence in Figures 1A-E.

[0571] The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.) The fragment then is digested with BglII and SpeI and again is purified on a 1% agarose gel. This fragment is designated "F1."

[0572] The plasmid is digested with the restriction enzyme Bam HI and XbaI and optionally can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). The vector DNA is designated herein "V1."

[0573] Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human TR16 gene using the PCR method, in which one of the primers that is used to amplify the gene and the second primer is from well within the vector so that only those bacterial colonies containing the TR16 gene fragment will show amplification of the DNA. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBacTR16.

[0574] 5µg of the plasmid pBacTR16 is co-transfected with 1µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofectin method described by Felgner *et al.*, *Proc. Natl. Acad. Sci. USA* 84:7413-7417 (1987). 1µg of BaculoGold™ virus DNA and 5µg of the plasmid pBacTR16 are mixed in a sterile well of a microtiter plate containing 50µl of serum free Grace's medium (Life Technologies, Inc., Rockville, MD). Afterwards, 10µl Lipofectin plus 90µl Grace's medium are added, mixed, and incubated for 15 minutes at room temperature. Then, the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35mm tissue culture plate with 1ml Grace's medium

without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours, the transfection solution is removed from the plate and 1ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

[0575] After four days, the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, cited above. An agarose gel with "Blue Gal" (Life Technologies, Inc., Rockville, MD) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies, Inc., Rockville, MD, pages 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200µl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-TR16.

[0576] To verify the expression of the TR16 gene, Sf9 cells are grown in Grace's medium supplemented with 10% heat inactivated FBS. The cells are infected with the recombinant baculovirus V-TR16 at a multiplicity of infection ("MOI") of about 2. Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies, Inc., Rockville, MD). If radiolabeled proteins are desired, 42 hours later, 5µCi of ³⁵S-methionine and 5µCi ³⁵S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled). Micro-sequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the mature protein and thus the cleavage point and length of the secretory signal peptide.

EXAMPLE 3

CLONING AND EXPRESSION OF THE TR16 RECEPTOR IN MAMMALIAN CELLS

[0577] A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g. RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular signals can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include, human Hela 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells, and Chinese hamster ovary (CHO) cells.

[0578] Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. Co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

[0579] The transfected gene can also be amplified to express large amounts of the encoded protein. The dihydrofolate reductase (DHFR) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy *et al.*, *Biochem. J.* 227:277-279 (1991); Bebbington *et al.*, *Bio/Technology* 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) cells are often used for the production of proteins.

[0580] The expression vectors pCl and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen *et al.*, *Molecular and Cellular Biology* 5:438- 447 (March 1985)), plus a fragment of the CMV-enhancer (Boshart *et al.*, *Cell* 41:521-530 (1985)).

Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat pre-pro-insulin gene.

EXAMPLE 3A

CLONING AND EXPRESSION OF THE EXTRACELLULAR SOLUBLE DOMAIN OF TR16 IN COS CELLS

[0581] The expression plasmid, pTR16-HA, is made by cloning a cDNA encoding TR16 into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.).

[0582] The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cell; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron, and a polyadenylation signal arranged so that a cDNA conveniently can be placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker.

[0583] A DNA fragment encoding the entire TR16 precursor and a HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson *et al.*, *Cell* 37:767 (1984). The fusion of the HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

[0584] The plasmid construction strategy is as follows:

[0585] Portions of the TR16 cDNA of the deposited clones is amplified using primers that contain convenient restriction sites, much as described above regarding the construction of expression vectors for expression of TR16 in *E. coli*.

[0586] To facilitate detection, purification and characterization of the expressed TR16, one of the primers contains a hemagglutinin tag ("HA tag") as described above.

[0587] Suitable primers for TR16 include the following, which are used in this example:

[0588] The 5' primer, 5'-GCAGCACATATGCTGTTCCGCGCCCGG-3' (SEQ ID

NO:40) contains the underlined BglII site, an ATG start codon and 5 codons thereafter. The 3' primer for TR16, which contains the underlined SpeI site, stop codon, hemagglutinin tag, and the last 20 nucleotides of the 3' coding sequence (at the 3' end), has the following sequence: 5'-CGCACTAGTTCAAGCGTAGTCTGGGACGTCGTATGG GTAGTTGAACAGATTCAAAATGG-3' (SEQ ID NO:41).

[0589] The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with BamHI and XbaI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis and gel sizing for the presence of the TR16-encoding fragment.

[0590] For expression of recombinant TR16, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook *et al.*, *Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, NY (1989). Cells are incubated under conditions for expression of TR16 by the vector.

[0591] Expression of the TR16-HA fusion protein is detected by radiolabelling and immunoprecipitation, using methods described in, for example Harlow *et al.*, *Antibodies: a Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ³⁵S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and then lysed with detergent-containing RIPA buffer: 150mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM TRIS, pH 7.5, as described by Wilson *et al.* cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE gels and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

EXAMPLE 3B

CLONING AND EXPRESSION OF TR16 USING THE CHO EXPRESSION SYSTEM

[0592] The vector pC4 is used for the expression of the TR16 polypeptide. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies, Rockville, MD) supplemented with the chemotherapeutic agent methotrexate (MTX). The amplification of the DHFR genes in cells resistant to MTX has been well documented (see, e.g., F.W. Alt *et al.*, *J. Biol. Chem.* 253:1357-1370 (1978); J.L. Hamlin and C. Ma, *Biochem. et Biophys. Acta* 1097:107-143 (1990); M.J. Page M.A. Sydenham, *Biotechnology* 9:64-68(1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained that contain the amplified gene integrated into one or more chromosome(s) of the host cell.

[0593] Plasmid pC4 contains, for expressing the gene of interest, the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen *et al.*, *Molecular and Cellular Biology* 5:438-447 (March 1985)), plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart *et al.*, *Cell* 41:521-530 (1985)). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI, XbaI, and Asp718. Behind these cloning sites, the plasmid contains the 3' intron and the polyadenylation site of the rat pre-pro-insulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human β -actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the TR16 polypeptide in a regulated way in mammalian cells. For the polyadenylation of the mRNA, other signals, e.g., from the human growth hormone or

globin genes, can be used as well.

[0594] Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418, or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

[0595] The plasmid pC4 is digested with the restriction enzyme BamHI and XbaI and then dephosphorylated using calf intestinal phosphates, by procedures known in the art. The vector is then isolated from a 1% agarose gel.

[0596] The DNA sequence encoding the complete TR16 polypeptide is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the desired portion of the gene.

[0597] The 5' oligonucleotide primer for TR16, containing the underlined BglII restriction site, a Kozak sequence, and an AUG start codon, has the sequence: 5'-GCAGCAAGATCTCCGCCATCATGCTGTTCCGCGCCCCGGGGGCCGGTAC-3' (SEQ ID NO:39).

[0598] The 3' primer for TR16, containing the underlined SpeI restriction site, has the sequence: 5'-GCAGCAACTAGTTTGTAGTCAACCGTTTCACAGGTTGCCAACTTTTC-3' (SEQ ID NO:37).

[0599] The amplified fragment is digested with BglII and SpeI and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. E. coli HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

[0600] Chinese hamster ovary cells lacking an active DHFR enzyme are used for transfection. 5µg of the expression plasmid pC4 are cotransfected with 0.5µg of the plasmid pSVneo using the lipofectin method (Felgner *et al.*, *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50ng/ml of MTX plus 1mg/ml G418. After about 10-14 days, single clones are trypsinized and then seeded in 6-well petri dishes or 10ml flasks

using different concentrations of methotrexate (50nM, 100nM, 200nM, 400nM, 800nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1μM, 2μM, 5μM, 10μM, 20μM). The same procedure is repeated until clones are obtained which grow at a concentration of 100-200μM. Expression of the desired gene product is analyzed, for instance, by Western blot analysis and SDS-PAGE, or by reversed phase HPLC analysis.

EXAMPLE 4

PROTEIN FUSIONS OF TR16

[0601] TR16 polypeptides of the invention are optionally fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of TR16 polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See EP A 394,827; Traunecker, *et al.*, *Nature* 331:84-86 (1988)). Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to TR16 polypeptides can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made using techniques known in the art or by using or routinely modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

[0602] Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence set forth below as SEQ ID NO:42. These primers also preferably contain convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

[0603] For example, if the pC4 (Accession No. 209646) expression vector is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and TR16 polynucleotide, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the

polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

[0604] If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891).

[0605] Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCACCGTGCCCAGC
ACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGAC
ACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGGTGGACGTAAGCC
ACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA
TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC
GTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG
TCTCCAACAAAGCCCTCCCAACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGG
GCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACC
AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCG
CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCC
CGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAG
AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
ACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGCGACG
GCCGCGACTCTAGAGGAT (SEQ ID NO:42)

EXAMPLE 5

PRODUCTION OF AN ANTIBODY

a) Hybridoma Technology

[0606] The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing TR16 are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of TR16 protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

[0607] Monoclonal antibodies specific for TR16 protein are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal

Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with TR16 polypeptide or, more preferably, with a secreted TR16 polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10g/l of nonessential amino acids, about 1,000U/ml of penicillin, and about 100µg/ml of streptomycin.

[0608] The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the TR16 polypeptide.

[0609] Alternatively, additional antibodies capable of binding to TR16 polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the TR16 protein-specific antibody can be blocked by TR16. Such antibodies comprise anti-idiotypic antibodies to the TR16 protein-specific antibody and are used to immunize an animal to induce formation of further TR16 protein-specific antibodies.

[0610] For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed infra. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature

312:643 (1984); Neuberger et al., Nature 314:268 (1985)).

b) Isolation Of Antibody Fragments Directed Against TR16 From A Library Of scFvs

[0611] Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against TR16 to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

[0612] Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10^9 *E. coli* harboring the phagemid are used to inoculate 50ml of 2xTY containing 1% glucose and 100µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. 5ml of this culture is used to inoculate 50ml of 2xTY-AMP-GLU, 2×10^8 TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100µg/ml ampicillin and 50µg/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

[0613] M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300ml 2xTY broth containing 100µg ampicillin/ml and 25µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2ml PBS and passed through a 0.45µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10^{13} transducing units/ml (ampicillin-resistant clones).

[0614] Panning of the Library. Immuntubes (Nunc) are coated overnight in PBS with 4ml of either 100µg/ml or 10µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10¹³ TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1ml of 100mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10ml of mid-log *E. coli* TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The *E. coli* are then plated on TYE plates containing 1% glucose and 100µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

[0615] Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect *E. coli* HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtiter plates coated with either 10pg/ml of the polypeptide of the present invention in 50mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing.

EXAMPLE 6

TISSUE DISTRIBUTION OF TR16 MRNA EXPRESSION

[0616] Northern blot analysis was carried out to examine TR16 gene expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the TR16 protein (SEQ ID NO:1) was labeled with ³²P using the *rediprime*TM DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe was purified using a CHROMA SPIN-100 column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe was then used to examine various human tissues for TR16 mRNA.

[0617] Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) were obtained from Clontech and were examined with labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots were mounted and exposed to film at -70°C overnight, and films developed according to standard procedures. Expression of TR16 was detected in tissues enriched in lymphocytes including peripheral blood leukocytes (PBLs), fetal liver, lung, kidney, small intestine, colon, keratinocytes, endothelial cells, and monocyte activated tissue. It can be envisaged that TR16 plays a role in lymphocyte homeostasis.

EXAMPLE 7

METHOD OF DETERMINING ALTERATIONS IN THE TR16 GENE

[0618] RNA is isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease). cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook et al.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:1. Suggested PCR conditions consist of 35 cycles at 95°C for 30 seconds; 60-120 seconds at 52-58°C; and 60-120 seconds at 70°C, using buffer solutions described in Sidransky, D., *et al.*, *Science* 252:706 (1991).

[0619] PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase (Epicentre Technologies). The intron-exon borders of selected exons of TR16 are also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations in TR16 is then cloned and sequenced to validate the results of the direct sequencing.

[0620] PCR products of TR16 are cloned into T-tailed vectors as described in Holton, T.A. and Graham, M.W., *Nucleic Acids Research*, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations in TR16 not present in unaffected individuals.

[0621] Genomic rearrangements are also observed as a method of determining alterations in the TR16 gene. Genomic clones isolated using techniques known in the art are nick-translated with digoxigenin deoxyuridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson, Cg. *et al.*, *Methods Cell Biol.* 35:73-99

(1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the TR16 genomic locus.

[0622] Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson, Cv. *et al.*, *Genet. Anal. Tech. Appl.*, 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region of TR16 (hybridized by the probe) are identified as insertions, deletions, and translocations. These TR16 alterations are used as a diagnostic marker for an associated disease.

EXAMPLE 8

METHOD OF DETECTING ABNORMAL LEVELS OF TR16 IN A BIOLOGICAL SAMPLE

[0623] TR16 polypeptides can be detected in a biological sample, and if an increased or decreased level of TR16 is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

[0624] For example, antibody-sandwich ELISAs are used to detect TR16 in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies to TR16, at a final concentration of 0.2 to 10 μ g/ml. The antibodies are either monoclonal or polyclonal and are produced using technique known in the art. The wells are blocked so that non-specific binding of TR16 to the well is reduced.

[0625] The coated wells are then incubated for >2 hours at RT with a sample containing TR16. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbound TR16.

[0626] Next, 50 μ l of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbound conjugate.

[0627] 75 μ l of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution is then added to each well and incubated 1 hour at room temperature to allow cleavage of the substrate and fluorescence. The fluorescence is measured by a microtiter plate reader. A standard curve is prepared using the experimental results from serial dilutions of a control sample with the sample concentration plotted on the X-axis (log scale) and fluorescence or absorbance on the Y-axis (linear scale). The TR16 polypeptide concentration in a sample is then interpolated using the standard curve based on the measured fluorescence of that sample.

EXAMPLE 9

METHOD OF TREATING DECREASED LEVELS OF TR16

[0628] The present invention relates to a method for treating an individual in need of a decreased level of TR16 biological activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of TR16 antagonist. Preferred antagonists for use in the present invention are TR16-specific antibodies.

[0629] Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of TR16 in an individual can be treated by administering TR16, preferably in a soluble and/or secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of TR16 polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of TR16 to increase the biological activity level of TR16 in such an individual.

[0630] For example, a patient with decreased levels of TR16 polypeptide receives a daily dose 0.1-100 μ g/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in a soluble and/or secreted form.

EXAMPLE 10

METHOD OF TREATING INCREASED LEVELS OF TR16

[0631] The present invention also relates to a method for treating an individual in need of an increased level of TR16 biological activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of TR16

or an agonist thereof.

[0632] Antisense technology is used to inhibit production of TR16. This technology is one example of a method of decreasing levels of TR16 polypeptide, preferably a soluble and/or secreted form, due to a variety of etiologies, such as cancer.

[0633] For example, a patient diagnosed with abnormally increased levels of TR16 is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the is determined to be well tolerated.

EXAMPLE 11

METHOD OF TREATMENT USING GENE THERAPY - EX VIVO

[0634] One method of gene therapy transplants fibroblasts, which are capable of expressing soluble and/or mature TR16 polypeptides, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37°C for approximately one week.

[0635] At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

[0636] pMV-7 (Kirschmeier, P.T. *et al.*, *DNA*, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

[0637] The cDNA encoding TR16 can be amplified using PCR primers which correspond to the 5' and 3' end encoding sequences respectively. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII

fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform *E. coli* HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector contains properly inserted TR16.

[0638] The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the TR16 gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the TR16 gene (the packaging cells are now referred to as producer cells).

[0639] Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether TR16 protein is produced.

[0640] The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

EXAMPLE 12

METHOD OF TREATMENT USING GENE THERAPY - IN VIVO

[0641] Another aspect of the present invention is using in vivo gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) TR16 sequences into an animal to increase or decrease the expression of the TR16 polypeptide. The TR16 polynucleotide may be operatively linked to a promoter or any other genetic elements necessary for the expression of the TR16 polypeptide by the target tissue. Such

gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata H. *et al.*, *Cardiovasc. Res.* 35:470-479 (1997); Chao J. *et al.*, *Pharmacol. Res.* 35:517-522 (1997); Wolff J.A. *Neuromuscul. Disord.* 7:314-318 (1997); Schwartz B. *et al.*, *Gene Ther.* 3:405-411 (1996); Tsurumi Y. *et al.*, *Circulation* 94:3281-3290 (1996) (incorporated herein by reference).

[0642] The TR16 polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The TR16 polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

[0643] The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the TR16 polynucleotides may also be delivered in liposome formulations (such as those taught in Felgner P.L., *et al.* *Ann. NY Acad. Sci.* 772:126-139 (1995), and Abdallah B., *et al.* *Biol. Cell* 85(1):1-7 (1995)) which can be prepared by methods well known to those skilled in the art.

[0644] The TR16 polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

[0645] The TR16 polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the

walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

[0646] For the naked TR16 polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.005mg/kg body weight to about 50mg/kg body weight. Preferably the dosage will be from about 0.005mg/kg to about 20mg/kg and more preferably from about 0.05mg/kg to about 5mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked TR16 polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

[0647] The dose response effects of injected TR16 polynucleotide in muscle *in vivo* is determined as follows. Suitable TR16 template DNA for production of mRNA coding for TR16 polypeptide is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

[0648] Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3ml of 2.5% Avertin. A 1.5cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The TR16 template DNA

is injected in 0.1ml of carrier in a 1cc syringe through a 27 gauge needle over one minute, approximately 0.5cm from the distal insertion site of the muscle into the knee and about 0.2cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

[0649] After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15µm cross-section of the individual quadriceps muscles is histochemically stained for TR16 protein expression. A time course for TR16 protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of TR16 DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using TR16 naked DNA.

EXAMPLE 13

GENE THERAPY USING ENDOGENOUS TR16 GENE

[0650] Another method of gene therapy according to the present invention involves operably associating the endogenous TR16 sequence with a promoter via homologous recombination as described, for example, in US Patent Number 5,641,670, issued June 24, 1997; International Publication Number WO 96/29411; International Publication Number WO 94/12650; Koller *et al.*, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); and Zijlstra *et al.*, *Nature* 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired. Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous TR16, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of TR16 so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the

amplified promoter.

[0651] The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

[0652] In this example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

[0653] Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous TR16 sequence. This results in the expression of TR16 in the cell. Expression may be detected by immunological staining, or any other method known in the art.

[0654] Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5ml of electroporation buffer (20mM HEPES pH 7.3, 137mM NaCl, 5mM KCl, 0.7mM Na₂HPO₄, 6mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3×10^6 cells/ml. Electroporation should be performed immediately following resuspension.

[0655] Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the TR16 locus, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two TR16 non-coding sequences are amplified via PCR: one TR16 non-coding sequence (TR16 fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other TR16 non-coding sequence (TR16 fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site

at the 3'end. The CMV promoter and TR16 fragments are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; TR16 fragment 1 - XbaI; TR16 fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

[0656] Plasmid DNA is added to a sterile cuvette with a 0.4cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120µg/ml. 0.5ml of the cell suspension (containing approximately 1.5×10^6 cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960µF and 250-300V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20mSec should be observed.

[0657] Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10cm dish and incubated at 37°C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

[0658] The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

EXAMPLE 14

BIOASSAY FOR THE EFFECT OF TR16 POLYPEPTIDES, AGONISTS, OR ANTAGONISTS ON HEMATOPOIETIC PROGENITOR CELLS AND/OR DIFFERENTIATION

[0659] Mouse bone marrow cells are used as target cells to examine the effect of TR16 polypeptides of the invention on hematopoietic progenitor cells and/or differentiation. Briefly, unfractionated bone marrow cells are first washed 2X with a serum-free IMDM that is supplemented with 10% (V/V) BIT (Bovine serum albumin, Insulin and Transferrin supplement from Stem Cell Technologies, Vancouver, Canada). The washed cells are then resuspended in the same growth medium and plated in the 96-

well tissue culture plate (5×10^4 cells/well) in 0.2ml of the above medium in the presence or absence of cytokines and TR16. Stem cell factor (SCF) and IL-3 are included as positive mediators of cell proliferation. Cells are allowed to grow in a low oxygen environment (5% CO₂, 7% O₂, and 88% N₂) tissue culture incubator for 6 days. On the sixth day, 0.5μCi of tritiated thymidine is added to each well and incubation is continued for an additional 16-18 hours, at which point the cells are harvested. The level of radioactivity incorporated into cellular DNA is determined by scintillation spectrometry and reflects the amount of cell proliferation.

[0660] The studies described in this example test the activity of TR16 polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16. Potential agonists would be expected to inhibit hematopoietic cell proliferation in the presence of SCF and/or IL3 and/or to increase the inhibition of cell proliferation in the presence of cytokines and TR16 in this assay. Potential antagonists would be expected to reduce the inhibition of cell proliferation in the presence of cytokines and TR16 in this assay.

EXAMPLE 15

BIOASSAY FOR THE EFFECT OF TR16 POLYPEPTIDES, AGONISTS OR ANTAGONISTS ON IL-3 AND SCF STIMULATED PROLIFERATION AND DIFFERENTIATION OF HEMATOPOIETIC PROGENITOR CELLS

[0661] To determine if TR16 polypeptides of the invention inhibit specific hematopoietic lineages, mouse bone marrow cells are first washed 2X with a serum-free IMDM that is supplemented with 10% (V/V) BIT (Bovine serum albumin, Insulin and Transferrin supplement from Stem Cell Technologies, Vancouver, Canada). The washed cells are then resuspended in the same growth medium and plated in the 96-well tissue culture plate (5×10^4 cells/well) in 0.2ml of the above medium in the presence of IL-3 (1ng/ml) plus SCF (5ng/ml) with or without TR16. Cells are allowed to grow in a low oxygen environment (5% CO₂, 7% O₂, and 88% N₂) tissue culture incubator, and after 7 days, analyzed for expression of differentiation antigens by staining with various monoclonal antibodies and FACScan.

[0662] The studies described in this example test the activity of TR16 polypeptides of

the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16. Potential agonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and TR16. Potential antagonists tested in this assay would be expected to reduce the inhibition of cell proliferation in the presence of cytokines and TR16.

EXAMPLE 16

EFFECT OF TR16 ON IL-3 AND SCF STIMULATED PROLIFERATION AND DIFFERENTIATION OF LIN-POPULATION OF BONE MARROW CELLS

A population of mouse bone marrow cells enriched in primitive hematopoietic progenitors can be obtained using a negative selection procedure, where the committed cells of most of the lineages are removed using a panel of monoclonal antibodies (anti cd11b, CD4, CD8, CD45R and Gr-1 antigens) and magnetic beads. The resulting population of cells (lineage depleted cells) are plated (5×10^4 cells/ml) in the presence or absence of TR16 polypeptide of the invention (in a range of concentrations) in a growth medium supplemented with IL-3 (5ng/ml) plus SCF (100ng/ml). After seven days of incubation at 37 °C in a humidified incubator (5% CO₂, 7% O₂, and 88% N₂ environment), cells are harvested and assayed for the HPP-CFC, and immature progenitors. In addition, cells are analyzed for the expression of certain differentiation antigens by FACSscan. Colony data is expressed as mean number of colonies +/- SD) and are obtained from assays performed in six dishes for each population of cells.

EXAMPLE 17

ASSAYS TO DETECT STIMULATION OR INHIBITION OF B CELL PROLIFERATION AND DIFFERENTIATION

[0663] Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been

found to influence B cell responsiveness including IL-2, IL-4, IL5, IL6, IL-7, IL10, IL-13, IL14 and IL15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations. One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

a) *In Vitro* assay

[0664] Purified TR16 polypeptides of the invention (e.g., soluble TR16) or agonists or antagonists thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of TR16 polypeptides, or agonists or antagonists thereof on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000ng/ml, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220). Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5×10^{-5} M β ME, 100U/ml penicillin, 10 μ g/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150 μ l. Proliferation or inhibition is quantitated by a 20h pulse

(1 μ Ci/well) with 3 H-thymidine (6.7Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

b) In Vivo Assay

[0665] BALB/c mice are injected (i.p.) twice per day with buffer only, or 2mg/Kg of TR16 polypeptide (e.g., soluble TR16) or agonists or antagonists thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal and TR16 polypeptide-treated spleens identify the results of the activity of TR16 polypeptide on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

[0666] Flow cytometric analyses of the spleens from TR16 polypeptide -treated mice is used to indicate whether TR16 polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

[0667] Likewise, a predicted consequence of increased mature B-cell representation *in vivo* is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and TR16 polypeptide-treated mice.

[0668] The studies described in this example test the activity in TR16 polypeptide. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), and agonists, and/or antagonists of TR16.

EXAMPLE 18

ASSAY FOR TR16 INHIBITION OF B CELL PROLIFERATION IN AN IN VITRO CO-STIMULATORY ASSAY

[0669] This example provides a co-stimulatory assay using *Staphylococcus Aureus* Cowan 1 (SAC) as priming agent and Neutrokin-alpha (International Application

Publication No. WO 98/18921) or IL-2 as a second signal to assay for TR16 polypeptide antagonists of Neutrokin-alpha (or IL-2) mediated B cell proliferation.

[0670] A soluble TR16 polypeptide is prepared (e.g., a soluble form of TR16 corresponding to a portion of the TR16 extracellular domain linked to the Fc portion of a human IgG1 immunoglobulin molecule). The ability of this protein to alter the proliferative response of human B cells is assessed in a standard co-stimulatory assay. Briefly, human tonsillar B cells are purified by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is routinely greater than 95% B cells as assessed by expression of CD19 and CD20 staining. Various dilutions of rHuNeutrokin-alpha (International Application Publication No. WO 98/18921) or rHuIL2 are placed into individual wells of a 96-well plate to which is added 10^5 B cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5×10^{-5} M 2-ME, 100U/ml penicillin, 10µg/ml streptomycin, and 10^{-5} dilution of formalin-fixed *Staphylococcus aureus* Cowan I (SAC) also known as Pansorbin (Pan)) in a total volume of 150µl. The TR16 polypeptide is then added at various concentrations and the plates are placed in the incubator (37°C 5% CO₂, 95% humidity) for three days. Proliferation is quantitated by a 20h pulse (1µCi/well) of ³H-thymidine (6.7Ci/mM) beginning 72h post factor addition. The positive and negative controls are SAC exposed B cells with rHuNeutrokin-alpha (or rHuIL2) and medium (in the absence of the TR16 polypeptide), respectively.

[0671] Antagonists of rHuNeutrokin-alpha (or rHuIL2) mediated B cell proliferation demonstrate a reduced level of B cell proliferation in the samples containing the TR16 polypeptides when compared to the positive control.

EXAMPLE 19

T CELL PROLIFERATION ASSAY

[0672] A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ³H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100µl/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4°C (1µg/ml in 0.05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5×10^4 /well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of

TR16 protein (total volume 200μl). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37°C, plates are spun for 2 min. at 1000 rpm and 100μl of supernatant is removed and stored -20°C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100μl of medium containing 0.5μCi of ³H-thymidine and cultured at 37°C for 18-24 hr. Wells are harvested and incorporation of ³H-thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of TR16 proteins.

[0673] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 20

EFFECT OF TR16 ON THE EXPRESSION OF MHC CLASS II, COSTIMULATORY AND ADHESION MOLECULES AND CELL DIFFERENTIATION OF MONOCYTES AND MONOCYTE-DERIVED HUMAN DENDRITIC CELLS

[0674] Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50ng/ml) and IL-4 (20ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF-α, causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FCγRII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

[0675] FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of TR16 or LPS (positive control), washed with PBS containing 1% BSA and 0.02mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4°C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan

(Becton Dickinson).

[0676] Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells (10^6 /ml) are treated with increasing concentrations of TR16 for 24 hours. LPS (100ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

[0677] Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

[0678] FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of TR16 or LPS (positive control), washed with PBS containing 1% BSA and 0.02mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4°C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

[0679] Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. TR16, agonists, or antagonists of TR16 can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

a) Monocyte Survival Assay

[0680] Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2×10^6 /ml in PBS containing PI at a final concentration of 5µg/ml, and then incubated at room temperature for 5 minutes before FAC Scan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

b) Effect on cytokine release

[0681] An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of 5×10^5 cells/ml with increasing concentrations of TR16 and under the same conditions, but in the absence of TR16. For IL-12 production, the cells are primed overnight with IFN-γ (100U/ml) in presence of TR16. LPS (10ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-α, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g., R & D Systems (Minneapolis, MN)) applying the standard protocols provided with the kit.

c) Oxidative burst

[0682] Purified monocytes are plated in 96-well plate at $2-1 \times 10^5$ cell/well. Increasing concentrations of TR16 are added to the wells in a total volume of 0.2ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2ml per well of phenol red solution (140mM NaCl, 10mM potassium phosphate buffer pH 7.0, 5.5mM dextrose, 0.56mM phenol red and 19U/ml of HRPO) is

added, together with the stimulant (200nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20µl 1N NaOH per well. The absorbance is read at 610nm. To calculate the amount of H₂O₂ produced by the macrophages, a standard curve of a H₂O₂ solution of known molarity is performed for each experiment.

[0683] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 21

THE EFFECT OF TR16 ON THE GROWTH OF VASCULAR ENDOTHELIAL CELLS

[0684] On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at 2-5x10⁴ cells/35mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16units/ml heparin, and 50units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8units/ml heparin. TR16 protein of SEQ ID NO:2, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter. An increase in the number of HUVEC cells indicates that TR16 may proliferate vascular endothelial cells.

[0685] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 22

STIMULATORY EFFECT OF TR16 ON THE PROLIFERATION OF VASCULAR ENDOTHELIAL CELLS

[0686] For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000

cells/well) in 0.1ml serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF₁₆₅ or TR16 in 0.5% FBS) with or without Heparin (8U/ml) are added to wells for 48 hours. 20mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al. In Vitro Cell. Dev. Biol.* 30A:512-518 (1994).

[0687] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 23

INHIBITION OF PDGF-INDUCED VASCULAR SMOOTH MUSCLE CELL PROLIFERATION STIMULATORY EFFECT

[0688] HAoSMC proliferation can be measured, for example, by BrdU incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6mg/ml BrdU. After 24 h, immunocytochemistry is performed by using BrdU Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdU antibody at 4°C for 2 h after exposing to denaturing solution and then with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdU-positive cells are counted. The BrdU index is calculated as a percent of the BrdU-positive cells to the total cell number. In addition, the simultaneous detection of the BrdU staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida *et al., J. Biol. Chem.* 6;271(36):21985-21992 (1996).

[0689] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of

TR16.

EXAMPLE 24

STIMULATION OF ENDOTHELIAL MIGRATION

[0690] This example will be used to explore the possibility that TR16 may stimulate lymphatic endothelial cell migration.

[0691] Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., Goodwin, R. H. J., and Leonard, E. J. "A 48 well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration." *J. Immunological Methods* 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 μ m (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 μ l of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber, 2.5×10^5 cells suspended in 50 μ l M199 containing 1% FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO₂ to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

[0692] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 25

STIMULATION OF NITRIC OXIDE PRODUCTION BY ENDOTHELIAL CELLS

[0693] Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, TR16 activity can be assayed by determining nitric oxide production by endothelial cells in response to TR16.

[0694] Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and TR16. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of TR16 on nitric oxide release is examined on HUVEC.

[0695] Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.). Calibration of the NO element is performed according to the following equation:



[0697] The standard calibration curve is obtained by adding graded concentrations of KNO_2 (0, 5, 10, 25, 50, 100, 250, and 500nmol/L) into the calibration solution containing KI and H_2SO_4 . The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas. The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) to maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per 1×10^6 endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al.* *Biochem. and Biophys. Res. Comm.* 217:96-105 (1995).

[0698] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of

TR16.

EXAMPLE 26

EFFECT OF TR16 ON CORD FORMATION IN ANGIOGENESIS

[0699] Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

[0700] CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 μ l/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 μ g Cell Applications' Chord Formation Medium containing control buffer or TR16 (0.1 to 100ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

[0701] Commercial (R&D) VEGF (50ng/ml) is used as a positive control. β -esteradiol (1ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

[0702] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 27

ANGIOGENIC EFFECT ON CHICK CHORIOALLANTOIC MEMBRANE

[0703] Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The ability of TR16 to stimulate angiogenesis in CAM can be examined.

[0704] Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese quail (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM

of 16-day-old chick and 13-day-old quail embryos is studied with the following methods.

[0705] On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5mm in diameter. Sterile and salt-free growth factors, and the protein to be tested, are dissolved in distilled water and about 3.3mg/ 5ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

[0706] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 28

ANGIOGENESIS ASSAY USING A MATRIGEL IMPLANT IN MOUSE

[0707] In order to establish an *in vivo* model for angiogenesis to test TR16 protein activities, mice and rats are implanted subcutaneously with methylcellulose disks containing either 20mg of BSA (negative control), 1mg of TR16, or 0.5mg of VEGF-1 (positive control). The negative control disks should contain little vascularization, while the positive control disks should show signs of vessel formation.

[0708] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 29

RESCUE OF ISCHEMIA IN RABBIT LOWER LIMB MODEL

[0709] To study the *in vivo* effects of TR16 on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously

(Takeshita, S. *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita, S. *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500µg naked TR16 expression plasmid by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen, R. *et al.*, *Hum Gene Ther.* 4:749-758 (1993); Leclerc, G. *et al.*, *J. Clin. Invest.* 90: 936-944 (1992)). When TR16 is used in the treatment, a single bolus of 500µg TR16 protein or control is delivered into the internal iliac artery of the ischemic limb over a period of 1 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number in the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

[0710] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 30

RAT ISCHEMIC SKIN FLAP MODEL

[0711] The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. TR16 expression, during the skin ischemia, is studied using in situ hybridization.

[0712] The study in this model is divided into three parts as follows:

Ischemic skin

Ischemic skin wounds

Normal wounds

[0713] The experimental protocol includes:

[0714] Raising a 3x4cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).

[0715] An excisional wounding (4-6mm in diameter) in the ischemic skin (skin-flap).

[0716] Topical treatment with TR16 of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100mg.

[0717] Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

[0718] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 31

PERIPHERAL ARTERIAL DISEASE MODEL

[0719] Angiogenic therapy using TR16 is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

[0720] One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.

[0721] TR16 protein, in a dosage range of 20mg - 500mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.

[0722] The ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of TR16 expression and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

[0723] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of

TR16.

EXAMPLE 32

ISCHEMIC MYOCARDIAL DISEASE MODEL

[0724] TR16 is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of TR16 expression is investigated *in situ*. The experimental protocol includes:

[0725] The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

[0726] TR16 protein, in a dosage range of 20mg - 500mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

[0727] Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and *in situ* analyzes.

[0728] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 33

RAT CORNEAL WOUND HEALING MODEL

[0729] This animal model shows the effect of TR16 on neovascularization. The experimental protocol includes:

[0730] Making a 1-1.5mm long incision from the center of cornea into the stromal layer.

[0731] Inserting a spatula below the lip of the incision facing the outer corner of the eye.

[0732] Making a pocket (its base is 1-1.5mm from the edge of the eye).

[0733] Positioning a pellet, containing 50ng- 5µg of TR16, within the pocket.

[0734] TR16 treatment can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

[0735] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the

activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 34

DIABETIC MOUSE AND GLUCOCORTICOID-IMPAIRED WOUND HEALING MODELS

a) Diabetic db+/db+ Mouse Model

[0736] To demonstrate that TR16 accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

[0737] The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*, *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

[0738] The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of Pathol.* 136:1235-1246 (1990)).

[0739] Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The

animals are purchased at 6 weeks of age and were 8 weeks old at the beginning of the study. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

[0740] Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01mg/ml), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

[0741] Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

[0742] TR16 is administered using at a range different doses of TR16, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50ml of vehicle solution.

[0743] Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

[0744] Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) TR16.

[0745] Wound closure is analyzed by measuring the area in the vertical and horizontal

axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 was 64mm², the corresponding size of the dermal punch. Calculations were made using the following formula:

[0746]
$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

[0747] Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with TR16. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

[0748] Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

[0749] Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer served as a positive tissue control and human brain tissue is used as a negative tissue control. Each specimen included a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

[0750] Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

b) Steroid Impaired Rat Model

[0751] The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, S.M. Glucocorticoids and Wound healing. In:

Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl, S.M. *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb, Z. *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert, R.H., *et al.*, *Am. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck, L.S. *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes, B.F. *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes, B.F., *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, S. M., "Glucocorticoids and wound healing", *In: Antiinflammatory Steroid Action: Basic and Clinical Aspects*, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck, L.S. *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes, B.F., *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, S. M., "Glucocorticoids and wound healing", *In: Antiinflammatory Steroid Action: Basic and Clinical Aspects*, Academic Press, New York, pp. 280-302 (1989); Pierce, G.F. *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

[0752] To demonstrate that TR16 can accelerate the healing process, the effects of multiple topical applications of TR16 on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

[0753] Young adult male Sprague Dawley rats weighing 250-300g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and were 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

[0754] The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50mg/kg) and xylazine (5mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile

gauze prior to wounding. An 8mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

[0755] Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue was no longer visible and the wound is covered by a continuous epithelium.

[0756] TR16 is administered using at a range different doses of TR16, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50ml of vehicle solution.

[0757] Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

[0758] Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) were evaluated: 1) Untreated group 2) Vehicle placebo control 3) TR16 treated groups.

[0759] Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 was 64mm^2 , the corresponding size of the dermal punch. Calculations were made using the following formula:

[0760]
$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

[0761] Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining was performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin was improved by treatment with TR16. A calibrated lens micrometer is used by a blinded observer to

determine the distance of the wound gap.

[0762] Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

[0763] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

EXAMPLE 35

LYMPHODEMA ANIMAL MODEL

[0764] The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of TR16 in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

[0765] Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

[0766] Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated or suture ligated.

[0767] Using a microscope, muscles in back of the leg (near the semitendinosus and

adductors) are bluntly dissected. The popliteal lymph node is then located.

[0768] The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

[0769] Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

[0770] To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

[0771] Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

[0772] Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

[0773] Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca^{2+} comparison.

[0774] Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs were amputated using a guillotine, then both experimental

and control legs were cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint was disarticulated and the foot was weighed.

[0775] Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at -80°C until sectioning. Upon sectioning, the muscle was observed under fluorescent microscopy for lymphatics. Other immuno/histological methods are currently being evaluated.

[0776] The studies described in this example test the activity in TR16 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of TR16 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of TR16.

[0777] The results of this experiment confirmed that TR16-Fc inhibited B cell proliferation in the co-stimulatory assay using *Staphylococcus Aureus* Cowan 1 (SAC) as priming agent and Neutrokin-alpha as a second signal (data not shown). It is important to note that other Tumor Necrosis Factor Receptors (TNFR) fusion proteins (e.g., DR4-Fc (International Application Publication No. WO 98/32856), TR6-Fc (International Application Publication No. WO 98/31799), and TR9-Fc (International Application Publication No. WO 98/56892)) did not inhibit proliferation.

[0778] It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

[0779] The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference.